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# BAG

## Journal of Basic & Applied Genetics

December 2022
Volume XXXIII
Issue 2
E-ISNN: 1852-6233

**Journal of the Argentine Society of Genetics**
Revista de la Sociedad Argentina de Genética

www.sag.org.ar/jbag
**Buenos Aires, Argentina**

DOI: 10.35407/bag.2022.33.02

Journal of the
Argentine Society
of Genetics

# BAG | Journal of Basic & Applied Genetics

V. XXXIII - No. 2

December 2022

Included in:

Cited by:

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**Imágen de tapa:**

Chororó *(Taraba major)* en el río Carcarañá. Casilda, Santa Fe.

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# VARIABILITY OF *PDYN* AND *OPRK1* GENES IN FOUR ARGENTINIAN POPULATIONS AND ITS GENETIC ASSOCIATION WITH CLINICAL VARIABLES RELATED TO ACUTE POSTSURGICAL PAIN

## VARIABILIDAD DE LOS GENES *PDYN* Y *OPRK1* EN CUATRO POBLACIONES ARGENTINAS Y SU ASOCIACIÓN CON VARIABLES CLÍNICAS RELACIONADAS AL DOLOR AGUDO POST-QUIRÚRGICO

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**Cite this article as:**
Di Santo Meztler G.P., Schiaffi J., Rigalli A., Esteban Torné M.E., Martina P.F., Catanesi C.I. 2022. VARIABILITY OF *PDYN* AND *OPRK1* GENES IN FOUR ARGENTINIAN POPULATIONS AND ITS GENETIC ASSOCIATION WITH CLINICAL VARIABLES RELATED TO ACUTE POSTSURGICAL PAIN. BAG. Journal of Basic and Applied Genetics XXXIII (2): 7-18.

Received: 08/20/2021
Revised version received: 02/04/2022
Accepted: 05/03/2022
General Editor: Elsa Camadro
DOI: 10.35407/bag.2022.33.02.01
ISSN online version: 1852-6233

Available online at
www.sag.org.ar/jbag

## ABSTRACT

Several population studies showed an association between variation in pain sensitivity and genetic polymorphisms located in Prodynorphin (*PDYN*) and Kappa Opioid Receptor (*OPRK1*) human genes. We analysed polymorphisms of these two genes to characterise their variation in Argentinian populations, as well as to evaluate their association with acute pain sensitivity. We studied 11 genetic markers in individuals from four locations in Argentina (Ciudad Autónoma de Buenos Aires, La Plata, Resistencia, and Misión Nueva Pompeya), calculated the population parameters, and evaluated the possible association among pain sensitivity, clinical, and genetic variables through a Generalised Estimating Equation model. High linkage disequilibrium was observed in the four populations for both genes, and significant differences were found among frequencies of Argentinian populations and those from other continents reported in the 1000 Genomes Project. Four *PDYN* gene polymorphisms from 3´ untranslated region and exon 4 showed association with acute pain sensitivity. One genotype of each of these polymorphisms was associated with a higher pain sensitivity, probably related with the activation of the N-methyl-D-aspartate (NMDA) receptors. We found a strong association with acute pain for the following clinical variables: 1) time after surgery, 2) intravenous klosidol supplied every 8 h, and 3) type of incision. Our results highlight the importance of a regional study of genetic variants which influence pain sensitivity and analgesic response.

Key words: human populations, pain sensitivity, acute pain, genetic polymorphisms, genetic structure

## RESUMEN

La asociación entre la sensibilidad al dolor y los polimorfismos que presentan los genes humanos de prodinorfina (*PDYN*) y receptor opioide kappa (*OPRK1*) se ha evidenciado en distintos estudios poblacionales. Con el objetivo de caracterizar la variación de estos genes y evaluar su asociación con dolor agudo en la población argentina, analizamos 11 polimorfismos en individuos provenientes de cuatro localidades argentinas (Ciudad Autónoma de Buenos Aires, La Plata, Resistencia, y Misión Nueva Pompeya). Calculamos los parámetros poblacionales y evaluamos la posible asociación entre sensibilidad al dolor, variables clínicas y variables genéticas a través de un modelo de ecuación generalizada de estimación. Se observó alto desequilibrio de ligamiento para ambos genes en las cuatro poblaciones analizadas, y se encontraron diferencias significativas entre las frecuencias de poblaciones argentinas y las reportadas en el Proyecto 1000 Genomes para poblaciones de otros continentes. Cuatro polimorfismos de la región 3´UTR y el exón 4 de *PDYN* mostraron asociación con la sensibilidad al dolor agudo. En cada uno de estos polimorfismos, un genotipo resultó asociado con alta sensibilidad al dolor, probablemente en relación con la activación de receptores N-metil-D-aspartato (NMDA). Encontramos una fuerte asociación con dolor agudo para las siguientes variables clínicas: 1) tiempo post-cirugía, 2) administración intravenosa de klosidol cada 8 h, y 3) tipo de incisión. Nuestros resultados resaltan la importancia de realizar estudios regionales de variables genéticas que influyen en la sensibilidad al dolor y la respuesta analgésica.

Palabras clave: poblaciones humanas, sensibilidad al dolor, dolor agudo, polimorfismos genéticos, estructura genética

## INTRODUCTION

The available information on the human genome gives a starting point for searching in different populations and among individuals for genome variability related to pain sensitivity and to the effectiveness of different drugs in pain relief (Owusu Obeng *et al.*, 2017; Crews *et al.* 2021). The human prodynorphin gene (*PDYN*), located on chromosome 20, encodes α-neoendorphin, β-neoendorphin, dynorphin A and dynorphin B. These molecules selectively activate kappa opioid receptors (KOR), encoded by *OPRK1* gene, which is located on chromosome 8 (Schwarzer, 2009; Hashemi *et al.*, 2018). Genetic association studies gave evidence of a link among certain DNA sequence variants of both genes and various pathologies, including cognitive disorders and drug abuse, as well as variations in pain sensitivity (Clarke *et al.*, 2012; Hashemi *et al.*, 2018; Nosova *et al.*, 2021).

It has been reported that an efficient management of postoperative acute pain is essential not only for improving the wellness of the patient, but also for reducing the risk of chronicity of pain, morbidity and mortality (Carr and Goudas, 1999). Genetic polymorphisms can explain some of the variation in response to analgesics, while other important variables also involved are the sex of the patient, the intensity and kind of pain, the environmental influences, and several psychological aspects including among others, anxiety and somatization (Stamer and Stüber, 2007; Schreiber *et al.*, 2014).

The challenge is to decipher the biological basis of such a complex phenotype, considering pain perception and response to analgesic drugs, both showing clear differences among populations of distinct origins. In Argentina, the current population is the result of several generations of intermixing among various groups at different times, including indigenous (Amerindian) communities, Spanish conquerors (early 1500s), Africans (arriving as slaves since the late 1500s until the end of slavery), and a large European immigrant population (arriving between 1870 and 1950) (Avena *et al.*, 2006). In this work we analyse four different populations from Argentina, namely from Ciudad Autónoma de Buenos Aires (CABA), La Plata, Resistencia, and Misión Nueva Pompeya (MNP).

### *Historical events*

Ciudad Autónoma de Buenos Aires (CABA) is the capital city of Argentina and it has by far the largest population in the country. During the second half of the 20th century, a significant demographic increment occurred in Argentina, mainly due to migratory flows (Gallo and Cortés Conde, 1967). From 1940 onwards, the industrial development encouraged people to move to CABA from other provinces of Argentina and from bordering countries bringing their indigenous genetic component (Torrado, 1992). While the European migratory contribution declined after 1930, immigrants from bordering countries are currently increasing the foreigner's contribution to the city (Avena *et al.*, 2001).

La Plata is the capital city of Buenos Aires province. Located 56 km southeast from CABA, it is the fourth most populous city in the country. As in CABA, an important European contribution in the past century is currently complemented by the arrival of populations from bordering countries looking for employment (Cerrutti, 2009).

In the case of Chaco province, it was originally inhabited by native people until 1528, when the first Europeans arrived. In 1872, a group of people from the province of Corrientes and Italian immigrants settled in this region. The city of Resistencia was then founded, and in 1884 it was assigned as the capital of the province of Chaco (De Pompert de Valenzuela, 2008; Tissera, 2008). At the end of the 19th century, European immigration to Resistencia was in order to promote urbanization and agricultural development (Maeder, 2012).

In 1900, the Franciscan missionaries founded the location of Misión Nueva Pompeya (MNP) in the western region of this province known as the *Impenetrable Chaqueño*. Currently, an important number of Native American people of the Wichí community still live in this inner region (Franceschi, 2010).

A previous report on the urban people living in MNP estimated a native contribution of 25% uniparental genetic markers (Sevini *et al.*, 2013). In fact, within the province of Chaco, Native American people from different communities live nearby several cities, and they still retain their traditional semi nomadic habits. The numerical importance of these native communities puts Chaco at present among the provinces with the highest number of living Native American people in Argentina (Instituto Nacional de Asuntos Indígenas, 2005).

Previous studies have shown genetic differences between these four populations using non coding X chromosome markers (SNPs, INDELs and STRs) (Di Santo Meztler, 2018; Di Santo Meztler *et al.*, 2019). Also, differences between a native Wichí community of Chaco and the population of Resistencia were found in the *OPRK1* gene (Raggio *et al.*, 2018).

In this work, our aim was to analyse whether interpopulation differences observed for non-coding genetic markers are also noticeable in coding regions, particularly for two genes of the opioid system and, therefore, to understand whether those differences have influence on the perception of pain. In particular, we focused on the genetic variability of *PDYN* and *OPRK1* in four Argentinian populations. Genetic association with clinical variants influencing pain sensitivity was

analysed for one of the populations, particularly after a surgical intervention.

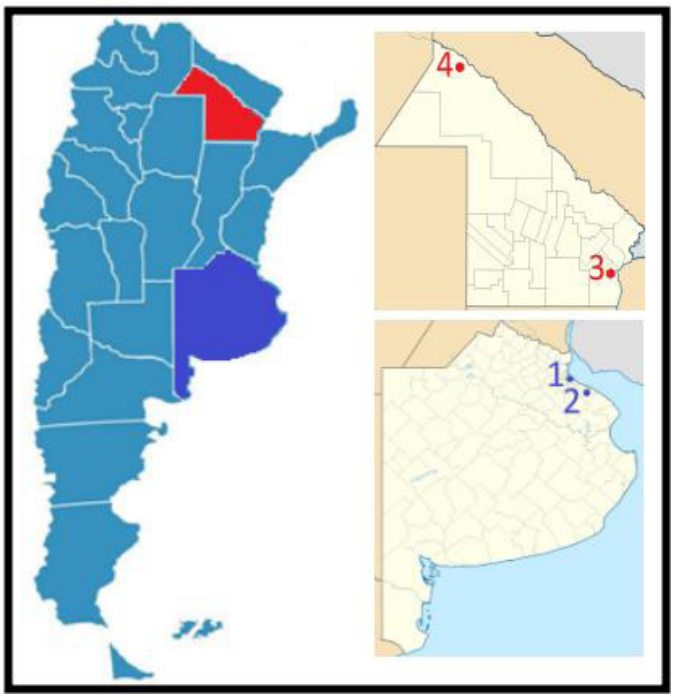


**Figure 1.** Location of the Argentinian populations analysed in this work. 1 = Ciudad Autónoma de Buenos Aires, 2 = La Plata, 3 = Resistencia, 4 = Misión Nueva Pompeya.

## MATERIALS AND METHODS

### *Populations*

Between 2009–2012 we collected a total of 286 samples from adult, unrelated persons from four different locations in Argentina: the capital city of Argentina, CABA (n=106), the capital city of Buenos Aires province, La Plata (n=33), the capital city of Chaco province, Resistencia (n=96), and a small city of Chaco province, MNP (n=54). Figure 1 shows a map indicating these locations. Samples from La Plata, Resistencia and MNP consisted of both male and female donors, and were collected during three field trips. Samples from CABA were only female donors, which were collected at Gynecology Service´s Breast Pathology Section of the Hospital General de Agudos Bernardino Rivadavia. The intensity of perceived pain and the requirement of analgesia after gynecological surgery were recorded for 50 out of 106 females from CABA. After discarding individuals with one or more missing data, the genetic association study was performed for a sample size of 35 females.

DNA was isolated from buccal and blood cells following protocols described in Gemmel and Akiyama (1996). *OPRK1* data for the population of Resistencia were previously reported in Raggio *et al.* (2018) and were included in this work for comparison. All biological samples were genotyped by author G. P. Di Santo Meztler at the Instituto Multidisciplinario de Biología Celular (IMBICE).

This study was part of a project previously approved by the Ethics Committee of the IMBICE, and all donors gave written consent for participation in the study.

### *Genetic determinations*

Eight polymorphisms (rs35286281, rs1997794, rs2235751, rs6045819, rs10485703, rs910080, rs910079, and rs2235749) were genotyped for *PDYN* gene, and three polymorphisms (rs35566036, rs3808627 and rs6985606) for *OPRK1* gene (Table 1). Genotyping was performed by PCR and separation of amplified fragments in 1.8% agarose gels, except for rs10485703, rs910080, rs910079 and rs2235749, which were amplified together in a fragment by PCR and then sequenced. For rs35286281 VNTR polymorphism, alleles were designated as 1(271pb), 2(339pb), 3(407pb) or 4(475pb) based on the number of repeated elements that were identified. Such elements contain a transcription factor binding site that is associated with transcriptional efficiency of the human *PDYN* gene, and higher gene expression is associated with repeated alleles 3 or 4 (Zimprich *et al.*, 2000). Therefore, the alleles 1 and 2 were categorised as low (L), and the alleles 3 and 4 as high (H) gene expression.

Primers for *PDYN* VNTR were obtained from Nikoshkov *et al.* (2008), primers for the SNPs located in the 3´ untranslated region (3´-UTR) of the gene were obtained from Yuferov *et al.* (2009), and primers for rs1997794, rs2235751 and rs6045819 were designed for this work (Supplementary Table 1).

Primers for *OPRK1* INDEL (rs35566036) were obtained from Edenberg *et al.* (2008) and allele specific primers for *OPRK1* SNPs were designed in our lab and reported in Raggio *et al.* (2018).

### *Statistical analysis*

#### *Population study*

For the genetic polymorphisms, allele frequencies were calculated using R v. 3.6.3 program (R Core Team, 2021). Heterozygosity, Hardy-Weinberg equilibrium (HWE) and genetic distance (as pairwise *Fst* values) were calculated through the program ARLEQUIN v.3.5 (Excoffier and Lischer, 2010), and linkage disequilibrium (LD) was calculated using the webtool SNPStats (Solé *et al.*, 2006) for obtaining both D´ and r values. In the case of repetitive polymorphism rs35286281, the alleles were pooled into short (271 and 339) and long (407 and

**Table 1.** Change and location of the analysed polymorphisms

| Gene/Marker | Change | Location |
|---|---|---|
| ***PDYN*** | | |
| rs35286281 | VNTR | promoter (1978756-1994285) |
| rs1997794 | C–T | 5´-UTR (1994212) a |
| rs2235751 | A–G | intron 2 (1989288) a |
| rs6045819 | A–G | exon 4 (1980488) a |
| rs10485703 | C–T | 3´-UTR (1979667) a |
| rs910080 | C–T | 3´-UTR (1979580) a |
| rs910079 | C–T | 3´-UTR (1979552) a |
| rs2235749 | C–T | 3´-UTR (1979293) a |
| ***OPRK1*** | | |
| rs35566036 | in–del | promoter (54328138: 54328137) |
| rs3808627 | A–G | promoter (53252242) a |
| rs6985606 | A–G | intron 2 (53248556) a |

a: Location according to NCBI (dsSNP - GRCh38)

**Table 2.** Clinical variables included in the analysis

| Clinical variable | Abbreviation | Levels |
|---|---|---|
| Time after surgery | Time | 1 h after<br>2 h after<br>12 h after<br>24 h after |
| Age of the patient | Age | - |
| Intravenous Klosidol | Ke8 | No<br>Yes |
| Associated pathologies | AP | No<br>Yes |
| Type of incision | I | Median Infraumbilical laparotomy (I1)<br>Pfannenstiel laparotomy incisions or breast surgery (I2)<br>Radian (I3)<br>Arcuate (I4)<br>Stewart mastectomy (Orr type) incision (I5) |

475) given that extreme alleles (271 and 475) are very infrequent, so that pooling them would produce no bias. The adaptation of the tables for R program and ARLEQUIN was made using GA-TA program (https://github.com/santimda/GA-TA) (Gamboa Lerena *et al.*, 2020). For population comparisons, data of four populations from 1000 Genomes database were included: Japanese in Tokyo (JPT); Mexican ancestry from Los Angeles (MXL), California, USA; residents of Utah with North and Western European ancestry (CEU) and Yoruba in Ibadan, Nigeria (YRI). Allele frequencies of these reference populations are detailed in Supplementary Table 2.

### *Association study*

For the association study we considered only females from the sample of CABA without missing data (n=35). We analysed the variation of pain informed by the physicians in a follow-up of 1, 2, 12 and 24 h after surgery; although the observations were informed by different surgeons, all pain reports were registered under supervision of author J. Schiaffi. For this analysis two models were evaluated: in one case the dependent variable was the pain scale reported by the physician, who considered wound palpation, analgesia requirement, possibility of walking, and pain escalation according to medical impression (model M); while in the other model the scale was reported by the patient according to self-perception (model P).

The independent variables used in both models were the above mentioned polymorphisms of *PDYN* and *OPRK1* genes (Table 1), and the clinical variables time after surgery, age of the patient, dose of intravenous Klosidol (Bagó Laboratory, Argentina) -which consists of a combination of dextropropoxyphene hydrochloride and dipyrone, supplied every 8 h according to pain intensity-, associated pathologies, and type of incision (Table 2). The type of incision depended on the type of surgery, which was either gynecological surgery (Median Infraumbilical laparotomy, and Pfannenstiel laparotomy incisions) or breast surgery (Radian, Arcuate, and Stewart mastectomy -Orr type- incisions). The analyses were made using R v.3.6.3, and GEE (Generalised Estimating Equation) model fitting was performed with the *geepack* library (Halekoh *et al.*, 2006).

## RESULTS

### *Population genetic analysis*

Allele frequencies for the *PDYN* and *OPRK1* polymorphisms are detailed in Table 3, and genotype frequencies in Supplementary Table 3. In the case of MNP, for rs35286281 the frequency of genotype 339/407 was higher than in the other populations, and for rs6045819 genotype G/G was absent. In none of the populations the genotype C/C was observed for rs10485703. In the case of Resistencia, for rs35566036 the frequency of genotype del/del was higher and that of the genotype in/del was lower than in the other populations.

For MNP, all the polymorphisms fitted HWE ($p$-value>0.05), but for the other three populations some of the markers did not fit HWE (Table 3). As we expected, linkage disequilibrium for *PDYN* gene was considerably high in all the populations. CABA and Resistencia showed higher LD than La Plata and MNP. For *OPRK1* the LD was lower in Resistencia and La Plata, while in CABA and MNP there were significant values of LD for all the markers (Supplementary Table 4).

**Table 3.** Allele frequencies and Hardy-Weinberg Equilibrium p-values for the polymorphisms analysed. For biallelic polymorphisms, one of the allele frequencies is showed.

| Gene | Locus | | CABA | | La Plata | | Resistencia [a] | | MNP | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Allele | freq. | p-value | freq. | p-value | freq. | p-value | freq. | p-value |
| | rs35286281 | 271 | 0.01 | 0.017 | 0.03 | 0.785 | 0.01 | 1.000 | -- | 0.408 |
| | | 339 | 0.36 | | 0.27 | | 0.34 | | 0.44 | |
| | | 407 | 0.62 | | 0.68 | | 0.64 | | 0.55 | |
| | | 475 | 0.01 | | 0.02 | | 0.02 | | 0.01 | |
| | rs1997794 | C | 0.42 | 0.016 | 0.31 | 0.296 | 0.39 | 0.829 | 0.42 | 0.577 |
| ***PDYN*** | rs2235751 | A | 0.68 | 0.000 | 0.66 | 0.689 | 0.72 | 0.456 | 0.66 | 1.000 |
| | rs6045819 | A | 0.87 | 0.368 | 0.88 | 0.048 | 0.91 | 1.000 | 0.94 | 1.000 |
| | rs10485703 | C | 0.15 | 1.000 | 0.05 | 1.000 | 0.09 | 1.000 | 0.07 | 1.000 |
| | rs910080 | C | 0.38 | 0.261 | 0.27 | 0.393 | 0.29 | 0.149 | 0.42 | 0.390 |
| | rs910079 | C | 0.37 | 0.251 | 0.27 | 1.000 | 0.29 | 0.093 | 0.40 | 0.564 |
| | rs2235749 | C | 0.62 | 0.259 | 0.74 | 0.638 | 0.69 | 0.055 | 0.60 | 0.545 |
| | rs35566036 | 300 | 0.75 | 1.000 | 0.66 | 1.000 | 0.88 | 0.000 | 0.67 | 0.375 |
| ***OPRK1*** | rs3808627 | A | 0.30 | 0.013 | 0.26 | 0.016 | 0.20 | 0.000 | 0.31 | 0.508 |
| | rs6985606 | A | 0.33 | 0.653 | 0.35 | 0.005 | 0.30 | 0.000 | 0.24 | 0.707 |

CABA= Ciudad Autónoma de Buenos Aires; MNP= Misión Nueva Pompeya
a: *OPRK1* data from Raggio *et al.* (2018)

The genetic distances were calculated for all the populations, and *Fst* with *p*-values lower than 0.05 were considered as significant (Tables 4 and 5). All four Argentinian populations showed significant differences with Africans from the Yoruba tribe in both genes, and also with Asians from Japan in *PDYN*, but differences in *OPRK1* resulted only significant for Resistencia. On the contrary, *OPRK1* resulted in significant values in the comparison with Europeans, while no differences were found when comparing with Mexicans for these two genes. Within Argentina, no differences were found for the SNPs comparisons (Table 4), whereas rs35566036 INDEL of *OPRK1* showed a differentiation among Resistencia and the other three Argentine populations, and rs5286281 VNTR of *PDYN* resulted significant only for CABA-MNP (Table 5).

## *Association Study*

### *GEE analysis*

Two GEE models were used to evaluate the association of the reported pain scale with the following variables: age of the patient (Age), dose of intravenous dextropropoxyphene hydrochloride + dipyrone every 8 hrs (Ke8), associated pathologies (AP), type of incision (I), time after surgery (Time) and the genotypes for each polymorphism. Model M was based on the pain scale reported by the physician (MScale) and Model P on the pain reported by the patient (PScale). Thus,

- Model M-->MScale(Time, Age, AP, Ke8, I, genetic polymorphisms)
- Model P-->PScale(Time, Age, AP, Ke8, I, genetic polymorphisms)

For this study we used 7 out of 11 genetic polymorphisms that fitted HWE (*p*-value>0.05). We only focused on these polymorphisms for the association study in order to avoid statistical artifacts of markers out of HWE, probably given by the sample size, so that we can reach an accurate association between genetic markers and pain. The ANOVA *p*-values obtained with the GEE models are shown in Table 6, where *p*-values<0.01 were considered as significant values.

After analysing the results of the previous GEE models, three clinical variables presented a strong association with pain (*p*-value<0.01) for the model M: Time, Ke8, and I, while for Model P only Time and I were significant. Regarding genetic polymorphisms, 5 out of 7 were significant for model M while 2 out of 7 resulted significant for Model P (Table 6A). Once the variables influencing pain sensation were identified, the analysis

**Table 4.** Pairwise genetic distances (*Fst* values) for SNP markers among the analysed populations and data from the 1000 Genomes Project. Above the diagonal: Fst values for OPRK markers; and below the diagonal: Fst values for PDYN markers. Significant Fst values (p-value <0.05) are highlighted in bold.

| Pairs of populations | CABA | La Plata | Resistencia | MNP | JPT[a] | MXL[a] | CEU[a] | YRI[a] |
|---|---|---|---|---|---|---|---|---|
| CABA | | -0.007 | 0.011 | 0.004 | -0.002 | -0.006 | **0.033** | **0.337** |
| La Plata | -0.010 | | -0.003 | 0.007 | 0.006 | -0.009 | 0.014 | **0.461** |
| Resistencia | -0.001 | -0.008 | | 0.015 | **0.031** | 0.010 | **0.038** | **0.256** |
| MNP | -0.006 | -0.012 | -0.005 | | 0.004 | 0.010 | **0.075** | **0.379** |
| JPT[a] | **0.280** | **0.317** | **0.326** | **0.305** | | 0.002 | **0.049** | **0.345** |
| MXL[a] | 0.001 | -0.007 | -0.006 | -0.004 | **0.351** | | **0.018** | **0.387** |
| CEU[a] | 0.005 | -0.002 | -0.003 | 0.001 | **0.360** | -0.006 | | **0.401** |
| YRI[a] | **0.280** | **0.289** | **0.327** | **0.302** | **0.148** | **0.341** | **0.356** | |

References: CABA = Ciudad Autónoma de Buenos Aires; MNP = Misión Nueva Pompeya; JPT = Japanese in Tokyo; MXL = Mexican ancestry from Los Angeles, California USA; CEU = Residents of Utah with North and Western European ancestry; YRI = Yoruba in Ibadian, Nigeria.
a: data from the 1000 Genomes Project

**Table 5.** Genetic differentiation (pairwise Fst values) for OPRK1 INDEL (rs35566036) and PDYN VNTR (rs35286281), among the analysed populations, and in comparison to data from other reports. Significant values (p<0.05) are in bold.

| Populations | ***OPRK1*** rs35566036 | | | | |
|---|---|---|---|---|---|
| | CABA | La Plata | Resistencia | MNP | Eur. Americans [a] |
| **CABA** | | -0.001 | **0.225** | 0.024 | **0.032** |
| **La Plata** | -0.002 | | **0.346** | -0.017 | **0.099** |
| **Resistencia** | 0.008 | -0.011 | | **0.394** | **0.119** |
| **MNP** | **0.039** | 0.03 | 0.007 | | **0.141** |
| **Germans** [b] | 0.009 | -0.015 | -0.008 | 0.013 | |
| | ***PDYN*** rs35286281 | | | | |

References: [a]: OPRK1 INDEL results were compared to non-Hispanic European Americans from Edenberg et al. (2008)
[b]: PDYN VNTR results were compared to Germans from Zimprich et al. (2000)

was focused within each level of the clinical variables and polymorphisms, taking into account one level per variable as a reference.

The reference level were 1 h after surgery (Time1), no analgesia rescue (Ke8(No)), Median Infraumbilical Laparotomy (I1), and no associated pathology (AP(No)), which were set at a coefficient value of zero (Table 6B). Thus, negative coefficients indicate pain decrease in comparison to the reference case. Postsurgical acute pain intensity was found to decrease as time increased in hours in both models, showing negative coefficients: Time 2(-1), Time 12(-1.556), Time 24(-2.259) for model M. The action of analgesia rescue (Ke8Yes) resulted in the same direction, showing a high negative coefficient.

Additionally, an ANOVA analysis was performed considering Time separately for one surgical incision at a time. ANOVA results for the first and second hours after the intervention were non-significant, but differences emerged for the reports at 12 and 24 h after incision (data not shown). The Pfannenstiel, Radian, Arcuate, and Orr surgery incisions showed a higher decrease of pain scale, with respect to the reference type of incision (Median Infraumbilical Laparotomy), being Pfannenstiel, Radian and Orr, the types of incision that presented the most important decrease in pain scale in both models.

Regarding genetic polymorphisms, in Model M the associated genotypes with lower pain sensitivity were rs6045819-A/G, rs10485703-C/T, rs910080-C/C, rs910079-C/C, and rs2235749-T/T, while for Model P the associated genotypes with lower pain sensitivity were rs6045819-A/G and rs35566036-del/del.

**Table 6 A.** ANOVA p-values obtained for two models of Generalised Estimating Equation. Model M uses the pain scale reported by the physician as a dependent variable, while Model P uses the pain scale reported by the patient. Significant p-values (p<0.01) indicate the clinical variables and/or genetic variants that influence pain susceptibility.

| Type of variable | Variables | Model M p-value | Model P p-value |
|---|---|---|---|
| Clinical variables | Time | **~0** | **0.0001** |
| | Age | 0.0268 | 0.0336 |
| | AP | 0.0104 | 0.0155 |
| | Ke8 | **0.0009** | 0.0234 |
| | I | **~0** | **~0** |
| PDYN | rs6045819 | **0.009** | **0.009** |
| | rs10485703 | **0.0058** | 0.0565 |
| | rs910080 | **0.006** | 0.2713 |
| | rs910079 | **0.0036** | 0.1247 |
| | rs2235749 | **0.006** | 0.2713 |
| OPRK1 | rs35566036 | 0.0436 | **0.0093** |
| | rs6985606 | 0.389 | 0.0698 |

*p*-values <0.01 were considered as significant values.

**Table 6 B.** Influence of clinical variables (Time, Ke8, I, AP and Age) on the pain scale reported by the physician or the patient. Coefficients, deviation standard and p-values of the generalised estimating equation (GEE). For each variable, the influence of the levels in the sensitivity to pain is shown. Negative coefficients for a level indicates lower pain respect to the reference level (coefficient for reference equals to zero).

| | Model M | | | Model P | | | |
|---|---|---|---|---|---|---|---|
| **Levels** | **Coefficient** | **S.E.** | **p-value** | **Coefficient** | **S.E.** | **p-value** | **Influence** |
| **(Intercept)** | 7.412 | 0.897 | ~0 | 8.031 | 0.884 | ~0 | |
| **Time1** | 0 | — | — | 0 | — | — | |
| **Time2** | -1.000 | 0.453 | 0.027 | -1.000 | 0.592 | 0.091 | - |
| **Time12** | -1.556 | 0.442 | ~0 | -1.778 | 0.553 | 0.001 | - |
| **Time24** | -2.259 | 0.401 | ~0 | -2.519 | 0.520 | ~0 | - |
| **Age** | -0.046 | 0.018 | 0.010 | -0.041 | 0.023 | 0.070 | - |
| **AP (No)** | 0 | — | — | 0 | — | — | |
| **AP (Yes)** | 0.533 | 0.385 | 0.166 | 0.482 | 0.461 | 0.296 | + |
| **Ke8(No)** | 0 | — | — | 0.000 | — | — | |
| **Ke8(Yes)** | -1.895 | 0.792 | 0.017 | -1.934 | 0.817 | 0.018 | - |
| **I1** | 0 | — | — | 0 | — | — | |
| **I2** | -1.350 | 0.485 | 0.005 | -1.739 | 0.632 | 0.006 | - |
| **I3** | -1.196 | 0.500 | 0.017 | -1.868 | 0.553 | 0.001 | - |
| **I4** | -1.071 | 0.698 | 0.125 | -1.447 | 0.868 | 0.096 | - |
| **I5** | -1.256 | 0.542 | 0.020 | -1.783 | 0.598 | 0.003 | - |
| **rs6045819(A/G)** | 0 | — | — | 0 | — | — | |
| **rs6045819(A/A)** | 1.057 | 0.345 | 0.002 | 0.917 | 0.402 | 0.023 | + |
| **rs6045819(G/G)** | 1.087 | 0.660 | 0.100 | 2.086 | 0.733 | 0.004 | + |
| **rs10485703(C/T)** | 0 | — | — | 0.000 | — | — | |
| **rs10485703(T/T)** | 0.9816 | 0.356 | 0.006 | 0.893 | 0.468 | 0.057 | + |
| **rs910080(C/T)** | 0 | — | — | 0 | — | — | |
| **rs910080(C/C)** | -0.8652 | 0.468 | 0.064 | -0.190 | 0.595 | 0.750 | - |
| **rs910080(T/T)** | 0.4394 | 0.340 | 0.196 | 0.524 | 0.435 | 0.228 | + |
| **rs910079(C/T)** | 0 | — | — | 0 | — | — | |
| **rs910079(C/C)** | -0.7167 | 0.485 | 0.139 | 0.079 | 0.576 | 0.891 | - |
| **rs910079(T/T)** | 0.5232 | 0.340 | 0.124 | 0.759 | 0.418 | 0.069 | + |
| **rs2235749(C/T)** | 0 | — | — | 0 | — | — | |
| **rs2235749(C/C)** | 0.4394 | 0.340 | 0.196 | 0.524 | 0.435 | 0.228 | + |
| **rs2235749(T/T)** | -0.8652 | 0.468 | 0.064 | -0.190 | 0.595 | 0.750 | - |
| **rs35566036(in/del)** | 0 | — | — | 0 | — | — | |
| **rs35566036(del/del)** | -0.83785 | 0.346 | 0.016 | -1.227 | 0.403 | 0.002 | - |
| **rs35566036(in/in)** | -0.39242 | 0.369 | 0.287 | -0.232 | 0.438 | 0.596 | - |
| **rs6985606(A/G)** | 0 | — | — | 0 | — | — | |
| **rs6985606(A/A)** | 0.2625 | 0.594 | 0.659 | -1.031 | 0.612 | 0.092 | + |
| **rs6985606(G/G)** | -0.4519 | 0.336 | 0.178 | -0.575 | 0.408 | 0.159 | - |

Reference levels: Time1, Ke8(No), AP(No), and I1.
S.E. = standard error

## DISCUSSION

The genetic basis influencing postoperative pain through the screening for variations in the expression of genes coding for endogenous opioid system is a field of interest for improving pain therapies (Stamer and Stüber, 2007; Montes *et al.*, 2015; Owusu Obeng *et al.*, 2017; Crews *et al.*, 2021).

In this work we analysed the genetic diversity of *PDYN* and *OPRK1* in Argentinian populations from different geographic locations, and the relationship between the genetic polymorphisms and the postsurgical pain, considering variables such as the use of analgesia and the different types of incisions.

Our results show that the genetic background of the Argentinian population differs in some aspects from that of other countries and continents (Avena *et al.*, 2006; Hohl *et al.*, 2018). In a previous report, the variation of *OPRM1* gene of the opioid system allowed to group Argentinians with other populations according to their ancestry, with 12.8% of differentiation among Africans, Asians, and European-Americans for this gene (López Soto and Catanesi, 2015).

Genetic differences have also been found among different regions or provinces within Argentina, for several genetic polymorphisms, whether they were coding or non-coding markers (Corach *et al.*, 2006; Avena *et al.*, 2012; Di Santo Meztler *et al.*, 2018; Muzzio *et al.*, 2018; Sala *et al.*, 2018; Caputo *et al.*, 2021, among others). Interestingly, some differences emerged even when comparing Resistencia and MNP, which are located in the same province and only 425 km apart, as it was previously reported for non-coding markers (Di Santo Meztler *et al.*, 2019). Our results showed less genetic differentiation among Argentinian populations, probably because the polymorphisms here analyzed are located in coding regions, having less chances for displaying variability. However, a significant Fst value was found between populations from CABA and MNP for the *PDYN* VNTR. This differentiation could be caused by the heterogeneous origin of immigrants from other continents, mainly Europeans from various countries who have settled in the past in particular locations along the territory of Argentina, as in CABA (Junta de Estudios Históricos del Municipio de Eldorado, 2015, 2016; Di Santo Meztler *et al.*, 2018). As opposed, MNP is somehow isolated due to hard weather conditions and floods that discourage immigration.

### *Clinical variables*

As several aspects of a surgical intervention can influence pain sensitivity, in this work we considered the interaction of both clinical variables and genetic variants.

When considering the evaluation of pain scale, the physician integrated the knowledge of the patient and the type of surgery performed, the questioning, the medical examination, and the data of the medical record (such as use of analgesics, calls to the nurse, rescue medication, etc.). Therefore, we considered that an evaluation by the professional (pain scale reported by the physician) is more trustworthy, and, in fact, significant associations of three clinical variables were found when analysing the medical pain scale.

Among the clinical variables to be taken into account, significant differences in reported pain scores were found according to the type of surgery. Although in the first two hours after the surgical intervention there were no differences between types of incision for pain scores, differences emerged as time increased, likely as a consequence of the severity of incision, being additionally influenced by biological factors as the genetic polymorphisms here analysed. Specifically, laparotomy incisions usually caused much higher pain scores on the first and second day after surgery than breast surgery incisions (either conservative or radical breast surgery). The results obtained in this work are in accordance with the grade of aggressivity of each incision. Pfannenstiel laparotomy involves the handling of the aponeurosis, thus giving a high pain sensation at the beginning but recovering faster in comparison to Radian, and even faster in comparison to Arcuate, and Orr incisions, which are less aggressive and usually cause a lower level of pain sensation from the beginning. On the contrary, Median laparotomy is the more aggressive, and the level of pain sensation likely persists more constantly along the first two days after surgery.

Concerning the analgesic rescues, in general Klosidol was known to be well-tolerated as analgesia of choice for postsurgical pain in Latin American populations from Bolivia and Argentina, with a good balance cost/benefit when it was prescribed for relief of postsurgical pain treatment (Daza Calderón *et al.*, 2010). However, this combination of dextropropoxyphene and dipyrone was discontinued more recently because of some serious adverse effects that were reported for European populations (ANMAT, 2008). As expected, the analgesic rescues indicated in our study had a significant effect in decreasing the pain sensation during the first hours after surgery.

### *Genetic variables*

Antinociception mediated by dynorphin and kappa receptors is known to be influenced by the sex of the patient, among other biological factors (Liu *et al.*, 2013). Moreover, the effect of certain *PDYN* polymorphisms has been reported showing sexual dimorphism, with a higher impact on females (Clarke *et al.*, 2012). Therefore, analysing genotype-phenotype association only in females avoids confounding results in this sense.

Among the genetic variants that we analysed, an association with pain sensitivity in the physician model was observed for one SNP (rs6045819) in the exon 4 and four SNPs (rs10485703, rs910080, rs910079, and rs2235749) in the 3´-UTR of *PDYN*. Genotypes associated with higher pain sensitivity were GG for rs6045819 and TT, TT, TT and CC respectively for 3´-UTR polymorphisms. There is evidence that exon 4 could be involved in *PDYN* splicing. This is supported by the significant association of the risk allele G (SNP rs6045819) with alcohol and/or cocaine dependence (Xuei *et al.*, 2006; Yuferov *et al.*, 2009). This risk allele could form a non-canonical E-box, which is a target of binding transcription factors that could modulate *PDYN* transcription, thus increasing the expression levels (Taqui, 2011). Regarding SNPs in the 3´-UTR of the gene, they are located close to each other, resulting in a significant LD among them (Supplementary Table 4). This genetic linkage is stronger among rs910080, rs910079 and rs2235749, likely transmitted in a block. This result is consistent with the finding that rs910079 can be chosen as a reporter of the block (Yuferov *et al.*, 2009). In addition, the haplotype rs910080-C /

rs910079-C / rs2235749-T has been proposed to be associated with a lower level of gene expression (Yuferov *et al.*, 2009).

Several works show that dynorphins inhibit nociceptive transmission in the spinal cord via interaction with the kappa opioid receptor (Werz and Macdonald, 1985; Randic *et al.*, 1995; Rusin *et al.*, 1997; Wiley *et al.*, 1997; Zachariou and Goldstein, 1997; Ogura and Kita, 2000). However, other authors have found evidence of dynorphin A having pronociceptive functions (Draisci *et al.*, 1991; Dubner and Ruda, 1992; Riley *et al.*, 1996; Vanderah *et al.*, 1996*et al., 1996*; Wagner and Deleo, 1996; Laughlin *et al.*, 1997; Malan *et al.*, 2000; Laughlin *et al.*, 2001). The switch between anti or pronociceptive effects of dynorphin A may depend on peptide concentrations, and kinetics of peptide interactions with either opioid or NMDA (N-methyl-D-aspartate) receptors. Dynorphins at physiological concentrations may be antinociceptive through the opioid receptors, typically playing an inhibitory role in acute pain conditions, whereas elevated pathophysiological levels may be pronociceptive and can interact with the NMDA receptors (Hauser *et al.*, 1999; Tan-No *et al.*, 2009). During peripheral inflammation, dynorphin induces its own synthesis through interaction with NMDA receptors, generating a regenerative, feed-forward process (Laughlin *et al.*, 2001).

In our work, we found genotypes that are associated with a high pain sensitivity and, according to bibliography, induce the expression of *PDYN*. We suggest that an overexpression of *PDYN* after surgery, in particular in patients with these genotypes, is giving rise to an activation of NMDA receptors, causing increased sensitivity to pain.

Concerning *OPRK1* gene, due to its wide presence in the central nervous system, its expression has been related to pain perception and behavioral traits as depression and drug abuse (Edenberg *et al.*, 2008; Bruchas *et al.*, 2010). The INDEL of *OPRK1*, rs35566036, was nearly significant for the model M. Different authors arrived to dissimilar conclusions on the importance of this polymorphism, either reporting a lack of association of this INDEL with the requirement of analgesia (Chatti *et al.*, 2017), or finding a regulatory effect on gene expression *in vitro* for the longer allele (insertion), thus acting as a transcriptional promoter with effect on a complex phenotype of alcohol dependence (Edenberg *et al.*, 2008). Our results are not conclusive for this matter, although the *p*-value near significance is suggestive of an influence of *OPRK1* INDEL on pain sensitivity. Increasing the sample number is probably needed in order to obtain a more accurate result.

Several previous reports on *PDYN* and *OPRK1* variation refer in general to dependence either on alcohol or drugs of abuse, given the abundance of dynorphin and kappa receptors on brain connections related to the formation of habits (Edenberg *et al.*, 2008; Zhang *et al.*, 2008; Dahl *et al.*, 2018; Hashemi *et al.*, 2018) and to emotional processing (Xu *et al.*, 2013). Other reports consider the influence of *PDYN* and *OPRK1* variants on pain modulation, mainly focused on chronic pain (Rosen *et al.*, 2000; Wang *et al.*, 2001; Podvin *et al.*, 2016; Tian *et al.*, 2018). Our results give an approach to the influence of the variation of both genes in pain, and suggest an association with levels of acute pain sensitivity and hyperalgesia after surgical intervention.

### *Concluding remarks*

This is the first report on Argentinian population for *PDYN* variation, while information available on *OPRK1* variation and pain sensitivity in the same population is scarce (Raggio *et al.*, 2018), an unfavorable scene given the geographic extent and the heterogeneity of Argentinian people.

The results presented in this work show differences between Argentinians and populations from other continents, even in the comparison to Europeans, suggesting that a component of admixture with Native American people probably reinforce the differences. This, however, cannot be confirmed due to the scarce available information for Native Americans on variation of the genes of the endogenous opioid system (Ehlers *et al.*, 1998; Raggio *et al.*, 2018) and other genes related to pain perception (Catanesi and Glesmann, 2015; López-Cortés *et al.*, 2020). For this reason, an analysis on other populations of the region with known admixture with Native communities is needed. Although the number of individuals included in the analysis needs to be further increased, a genetic association with postsurgical acute pain phenotype has been found.

These findings highlight the importance of a regional study of genetic variants influencing pain sensitivity and analgesic response, in tune with the current tendency of a personal therapy medicine.

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## ACKNOWLEDGEMENTS

This research was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina, PIP 2015-2017/0930), Agencia Nacional de Promoción Científica y Tecnológica (PICT -2020-SERIEA-01075), and from Universidad Nacional de La Plata (UNLP, Argentina, PID 2019-2020/N895). We would like to acknowledge Dr. Laura Angela Glesmann, MSc. Raúl Jorge Bridi and Dr. María Celeste Raggio for being part of the field trips for sample collection. We also thank Mr. Eduardo César Bauzá for the English language revision, and two anonymous reviewers of the manuscript.

BAG | Journal of Basic & Applied Genetics

Vol XXXIII (2): 19-25; December 2022

Journal of the Argentine Society of Genetics

# IMPACT OF GENETIC ANCESTRY ON THE DISTRIBUTION OF INTERFERON-λ4 RS12979860 POLYMORPHISM IN A GLOBAL POPULATION OF BUENOS AIRES, ARGENTINA

## IMPACTO DE LA ANCESTRÍA GENÉTICA EN LA DISTRIBUCIÓN DEL POLIMORFISMO DE INTERFERÓN-λ4 RS12979860 EN UNA POBLACIÓN GLOBAL DE BUENOS AIRES, ARGENTINA

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**Cite this article as:**
Mansilla F.C., Avena S.A., Dejean C.B., Turco C.S., Capozzo A.V. 2022. IMPACT OF GENETIC ANCESTRY ON THE DISTRIBUTION OF INTERFERON-λ4 RS12979860 POLYMORPHISM IN A GLOBAL POPULATION OF BUENOS AIRES, ARGENTINA. BAG. Journal of Basic and Applied Genetics XXXIII (2): 19-25.

Received: 03/04/2022
Revised version received: 06/30/2022
Accepted: 08/08/2022
General Editor: Elsa Camadro
DOI: 10.35407/bag.2022.33.02.02
ISSN online version: 1852-6233

Available online at
www.sag.org.ar/jbag

## ABSTRACT

Human interferon-λ4 is a cytokine involved in early stages of antiviral responses. Strikingly, some allelic variants with diminished antiviral activity reduce the susceptibility to viral infections, thus they would have suffered a positive selection pressure throughout the evolutionary history of the genus *Homo*. An intronic variant within the IFNλ4 locus (rs12979860, T>C) emerged as one of the main gene determinants of the response to HCV and other viruses. The rs12979860-C allele has a differential frequency in African, European and Native American populations, though South American data are scarce. Here we characterize for the first time the distribution of rs12979860 genotypes in a sample of the global population of Buenos Aires, Argentina, assessing its association with European, Native American and African parental components. The rs12979860 genotypes were determined by PCR-RFLP in DNA samples from donors of a blood banks of Buenos Aires (n=96), whose genetic individual ancestry (European, African or Native American) had been previously determined using molecular markers. The distribution of rs12979860-CC, CT and TT was 29.17%, 50.0% and 20.83%, respectively. A significant increase in the frequency of CC among donors with a strong European contribution and a greater impact of the Native American component among donors carrying the T allele were observed. Native American and European components were associated to the rs12979860 distribution in a sample of the global population of Buenos Aires, while no differences were directly attributable to the African ancestry. Considering interferon´s key role in antiviral responses, our results may contribute to both bioanthropological and immunogenetic studies associated with infectious diseases.

Key words: ancestry, Buenos Aires, IFNλ4 polymorphism, rs12979860 distribution.

## RESUMEN

El interferón-λ4 humano es una citoquina involucrada en la respuesta antiviral. Algunas variantes alélicas con menor actividad antiviral, paradójicamente, reducen la susceptibilidad a infecciones virales, por lo que habrían sufrido una presión de selección positiva en la historia evolutiva del género *Homo*. Una variante dentro del locus de IFNλ4 (rs12979860, T>C), con distribución diferencial en poblaciones africanas, europeas y nativas americanas, surgió como uno de los principales determinantes genéticos de la respuesta al HCV y otros virus. Aquí caracterizamos por primera vez la distribución de los genotipos de rs12979860 en una muestra de la población cosmopolita de Buenos Aires, Argentina, evaluando el impacto de su ancesrtría. Se determinaron diferentes genotipos de rs12979860 por PCR-RFLP en muestras de ADN de donantes de bancos de sangre de Buenos Aires (n=96), cuya ancestría individual había sido previamente determinada mediante diferentes marcadores moleculares. La distribución global de rs12979860-CC, CT y TT fue 29,17%; 50,0% y 20,83%, respectivamente. Se observó un aumento significativo de la frecuencia del genotipo CC entre individuos con fuerte aporte europeo y un mayor impacto del componente nativo-americano entre portadores del alelo T. Los componentes nativo-americano y europeo se asociaron a la distribución rs12979860 en una muestra poblacional global de Buenos Aires, mientras que no se vieron diferencias directamente asociadas a la ancestría africana. Considerando el papel clave del interferón en la respuesta antiviral, nuestros resultados pueden contribuir a estudios con un enfoque bioantropológico así como a estudios inmunogenéticos asociados a enfermedades infecciosas.

Palabras clave: ancestría, Buenos Aires, polimorfismo en IFNλ4, distribución de rs12979860.

## INTRODUCTION

Lambda interferons (IFNλ) are cytokines rapidly produced by most vertebrates during the innate immune response, constituting the first line of defense against viral infections (Lazear *et al.*, 2015). IFNλ1, 2 and 3 were identified in 2003 (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003) and in 2013 a functional form of IFNλ4 was firstly characterized (Prokunina-Olsson *et al.*, 2013). The IFNλ4 locus (19q13.2) is highly polymorphic (Fang *et al.*, 2020) and it was reported that some allelic variants can modulate the susceptibility, progression and response to treatments against different viral infections (Chatterjee, 2010; Bravo *et al.*, 2014; Angulo *et al.*, 2015; Ispirologlu *et al.*, 2017; da Silva Cezar *et al.*, 2020). Interestingly, the most favorable alleles in this regard correspond to mutations that are in strong linkage disequilibrium and restrict the expression, stability or antiviral activity of IFNλ4 (Booth and George, 2013; O'Brien *et al.*, 2014; Prokunina-Olsson, 2019).

Throughout the evolutionary history of the genus *Homo* these mutations have suffered a positive selection pressure resulting in a differential global distribution which is correlated to the ancestry of different human populations and may affect the immune response to different pathogens (Key *et al.*, 2014; Bamford *et al.*, 2018). An intronic variant that reduces the antiviral activity of IFNλ4 (rs12979860, T>C) was characterized as the main gene determinant of the response against Hepatitis C Virus (HCV). The rs12979860-T allele is associated with lower sustained virologic response (SVR) rates and a lower percentage of treatment success (Ge *et al.*, 2009). On the other hand, the CC genotype was strongly associated with spontaneous resolution and lower susceptibility to HCV infection (Thomas *et al.*, 2009; Pedergnana *et al.*, 2012; Indolfi *et al.*, 2014a; Fan *et al.*, 2016). Moreover, genotyping of rs12979860 is recommended to predict the patient´s response to different antiviral treatments (Sharafi *et al.*, 2012; Ramamurthy *et al.*, 2018). Different correlations between rs12979860 and clinical phenotypes associated with other viral infections have also been reported, conditioning the susceptibility, evolution and/or response to treatment against Hepatitis B and D (Ispirologlu *et al.*, 2017), Dengue (da Silva Cezar *et al.*, 2020), HIV (Chatterjee, 2010; Zaidane *et al.*, 2018), CMV (Bravo *et al.*, 2014; Chmelova *et al.*, 2019) and coronaviruses (Hamming *et al.*, 2013).

The rs12979860-C allele has a global frequency of 0.23–0.55 in African populations; 0.53–0.80 for Europeans and 0.72–1.00 for Asians, with higher frequencies in eastern Asia. Data about the distribution of these variants in South American populations are scarce and tend to be biased due to the small sample size and the genetic admixture of the populations assessed.

The Argentinean population´s ancestry is the result of a deep miscegenation, product of different migratory waves during the last centuries, which means that the European, Native American and African components (frequently underestimated) are present at different degrees in the gene pool of different cosmopolitan populations of the country (Avena *et al.*, 2012). In this regard, the immunogenetic profiling of IFNλ4-rs12979860, and the association with its ancestry, may be a potential tool in both anthropological and biomedical studies associated with infectious diseases. The objective of this study was to determine the distribution of the allelic variants of rs12979860 in a cosmopolitan population of Buenos Aires, Argentina, whose ancestry had been previously determined by assessing a set of 106 biallelic SNPs (Ancestry Informative Markers) widely spaced and balanced throughout the genome, that can discriminate Native American, African and European ancestry (Avena *et al.*, 2012).

## MATERIALS AND METHODS

### *Study Design*

This study comprised DNA samples from unrelated donors from both public and private hospitals blood banks in Buenos Aires, Argentina (n=96). Informed consent was obtained from all individual participants included in the study. Most of them (89/96) also agreed to provide information about the region/country of birth of all their grandparents, which was included in the data analysis. The study was approved by the Ethics Committee of the Hospital Italiano of Buenos Aires and was performed in accordance with the ethical standards adopted in the Declaration of Helsinki.

### *rs12979860 genotyping*

Different genotypes of rs12979860 were determined by PCR-RFLP, as it was previously described (Sharafi *et al.*, 2012). A 241 bp fragment was amplified by endpoint PCR (Taq Pegasus®, Productos Bio-Lógicos, Bs. As., Argentina) following a standard cycle (5 min at 94° C; 35 cycles of 20 s at 94° C, 20 s at 59° C and 20 s at 72° C; and 5 min at 72° C) and then digested with Bsh12361 restriction enzyme (Thermo Fisher, DE, USA; 1U/reaction) for 1 h at 37° C. The primers used were 5´GCGGAAGGAGCAGTTGCGCT3´ (Fw) and 5´TCTCCTCCCCAAGTCAGGCAACC3´ (Rv) and the resulting fragments (rs12979860-CC = 196 + 45 bp; rs12979860-CT = 241 + 196 + 45 bp; rs12979860-TT = 241 bp) were revealed by agarose gel electrophoresis (3%) stained with GelRed (Biotium, CA, USA).

### *Statistical analysis*

The allelic frequencies were determined, and Hardy-Weinberg equilibrium was assessed using the chi-square test (Microsoft Excel GenAIEx 6.5, Peakall and Smouse, 2012) to compare the genotype distribution. Differences associated to European, Native American or African component were determined using T test (GraphPad Prism 9). In all statistical analysis a $p<0.05$ was considered as statistically significant and $\alpha=0.05$ was set as the risk level.

## RESULTS AND DISCUSSION

The average individual ancestry was estimated as 69.4% European, 26.3% Native American and 4.3% African. Frequencies lower than 0.02 were not included in the data analysis since they may be associated to technical artifacts. The European component was present in every tested sample, with individual frequencies ranging from 0.02 to 1. The Native American component was also detected but to a lesser extent, in 79% of the samples (frequencies 0.02-0.8). Finally, the African ancestry was detected in 41% of the samples, with a frequency range from 0.02 to 0.23 (Figure 1, modified from Avena *et al.*, 2012). This evidences the multiplicity of origins of Buenos Aires´ population, resulting of the miscegenation between Native Americans, enslaved Africans who came mainly from West Africa and Mozambique until the first half of the 19$^{th}$ century (Fejerman *et al.*, 2005) and European immigrants, mainly from Italy and Spain, who arrived in the country between 1870 and 1960 (Avena *et al.*, 2006; Muzzio *et al.*, 2018). These results are in line with previously published data (Avena *et al.*, 2006), further challenging the European self-perception as Argentina's identity.

Several studies have reported the distribution of the rs12979860 genotypes in different populations, mainly assessing its correlation with the susceptibility to different viral infections and response to antiviral treatments (Wu *et al.*, 2012; Porto *et al.*, 2015; Taheri *et al.*, 2015; Echeverría *et al.*, 2018). The correlation of this distribution and the local ancestry of these populations as well as its implications have also been assessed (Indolfi *et al.*, 2014b; Rizzo *et al.*, 2016), though this is the first report in an Argentinean global population. The overall distribution of rs12979860-CC, CT and TT was 29.17%, 50.0% and 20.83%, respectively. Hardy-Weinberg equation was used to calculate the genetic variation of this population at equilibrium. Significant differences were not detected (chi-square test: 0.00469; $p=0.99766$), thus suggesting that the impact of posible microevolutionary mechanisms and population structure is not significant. The allelic frequencies for C and T were 54.17% and 45.83%, respectively. These results differ from data reported in HCV chronically infected patients of a public center in Buenos Aires, with an allelic frequency of C=0.6 and 45.0% of heterozygosity (Machicote *et al.*, 2018). This higher frequency of rs12979860-C is expected as it is known that this allele is favorable in both acute and chronic HCV infection. In this regard, the differences observed between healthy and infected individuals highlight the impact of assessing global populations when studying the distribution of this kind of markers.

A significant increase in the frequency of CC genotype was observed among donors with a strong European contribution (Figure 2a, $p<0.05$). Our results also suggest a greater impact of the Native American component among donors carrying the T allele (both CT and TT genotypes), although differences were marginally significant (Figure 2b). No differences in the rs12979860 distribution were directly attributable to the African component (Figure 2c, $p>0.05$), represented at low levels in our sample.

Based on previously reported data on the composition and immigration patterns of the admixed population of Buenos Aires (Avena *et al.*, 2012), we defined our parental population including sub-Saharan Africans (involved in slavery trafficking) and Europeans from Italy and Spain (Avena *et al.*, 2006). To minimize bias, we only considered

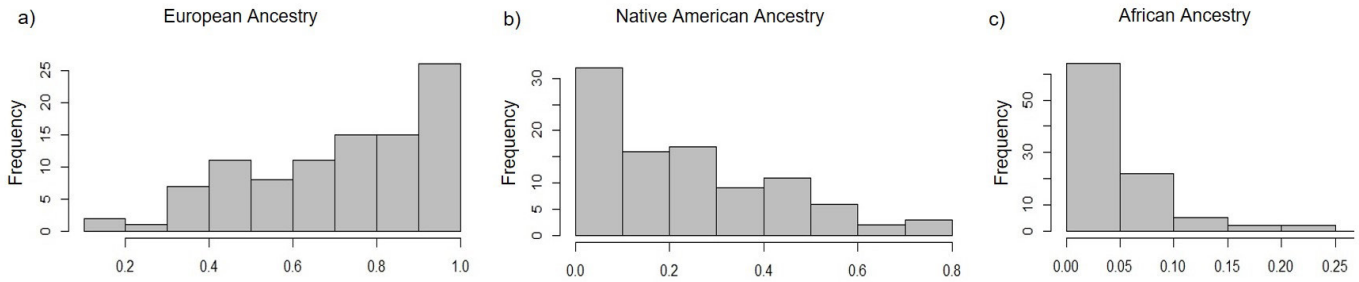


**Figure 1.** Frequency distribution of the individual European (a), Native American (b) and African ancestry (c) among healthy donors from Buenos Aires, Argentina, enrolled in this study (n=96). Modified from Avena *et al.* (2012).

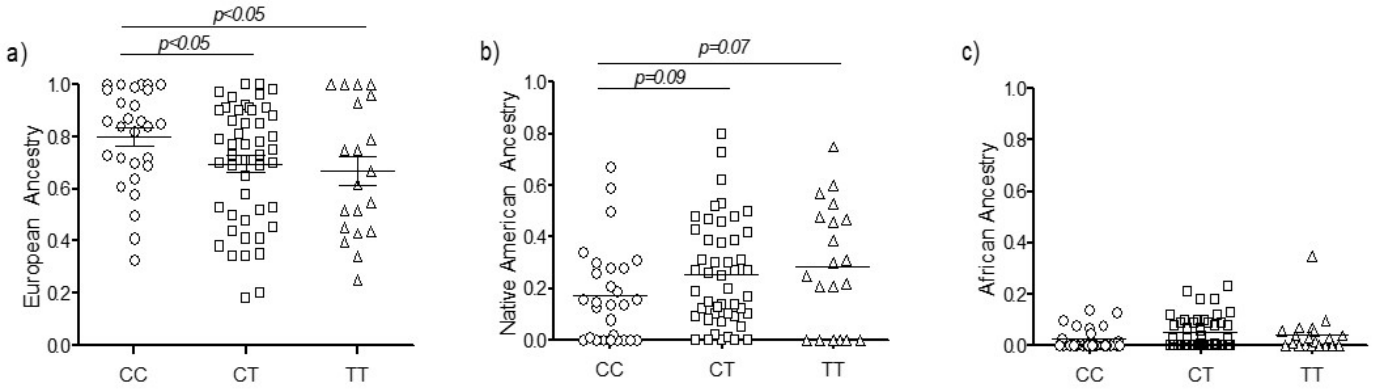


**Figure 2.** Distribution of European (a), Native American (b) and African ancestry (c) among individuals carrying rs12979860-CC, CT and TT genotypes. $p<0.05$ were considered as statistically significant.

reported data on the rs12979860 distribution (Table S1) from non-cosmopolitan populations with a sample size greater than 50. Ethiopian Jews and Sephardic Jews from Rome, Italy, were also excluded, as these groups tend to be endogamous and have a different origin, which may introduce certain bias to our analysis. The mean frequency of the rs12979860-C allele for this parental population is 0.654 for Europeans, 0.298 for Africans and 0.518 for Native Americans (table S1). However, data available regarding the Native American component are scarce and are often based either on cosmopolitan admixed populations or studies with very small sample sizes and variable results. Despite the lack of a robust sample to perform comparisons, our results suggest that populations with greater autochthonous ancestry tend to exhibit higher frequencies of the rs12979860-T allele.

Further studies are needed to fully characterize the distribution of this polymorphism in Latin America, as available data seem to be contradictory. To explain this it is important, regarding cosmopolitan populations, to disclose their composition and their genetic ancestry in order to determine their parental populations´contribution. Latin American cosmopolitan populations are known to be admixed, but the European, Native American and sub-Saharan contributions have marked regional differences. Hence the relevance of studying cases such as the one here described considering the genetic ancestry of the population under study.

The frequency of rs12979860-C in Buenos Aires´ individuals was similar to previously reported data for populations from Tuscany (C=0.603) in Italy, which are among the lowest compared to other West European populations (Table S1). The reported frequency of this allele in an Iberian population, however, was higher than the one described in our study (C=0.705, Table S1). Although immigrants from both Italy and Spain are the main determinants of the European ancestry of Buenos Aires´ population (Avena *et al.*, 2006), it is to note that most of the immigrants in Buenos Aires (and Argentina) were of Italian origin (Avena *et al.* 2006). This may explain, at least partially, the frequencies here described.

In order to further characterize the European contribution to the rs12979860 distribution we considered, when available, the self-reported data about grandparents' origins. Interestingly, a total of 53 individuals declared the nonexistence of grandparents of European origin (8/53:CC, 33/53:CT and 12/53:TT), while only 36 individuals reported at least one grandparent from Italy, Spain/Portugal or other European countries (14/36:CC, 16/36:CT and 6/36:TT). This may be attributed to the fact that the vast majority of immigrants arrived in Buenos Aires before 1950. In our sample, the presence of Iberian ancestry seems to be underrepresented, as genealogical data suggest that the self-reported Italian ancestry was 33.0% higher than Iberian ancestry. Altogether, our results may be explained by the higher presence of Italian ancestry among European descendants in our sample, as well as by the admixture of these individuals with Native Americans and Africans or afro-descendants with a higher rs12979860-T frequency, thus increasing the heterozygosity and the rs12979860-T frequency. However, it is important to consider that, despite being very useful especially in regions with recent immigration patterns (Avena *et al.* 2012), this kind of surveys must be carefully analyzed, since different social and economic aspects may influence the individual self-perceived ancestry, as it was recently reported (Paschetta *et al.* 2021).

Notably, most of the populations that have been included in large-scale immunogenomic studies were

of European origin, and might include certain bias by demographic, social and economic conditions of non-randomly selected individuals (Peng *et al.*, 2021). This may have affected the representativeness of the sample, thus compromising the conclusions of those studies. Therefore, increasing the genetic diversity while considering these structural inequalities is mandatory in order to obtain more reliable results. The PCR-RFLP protocol here applied was previously described and fully validated against PCR-sequencing, with a concordance of 100% in the results obtained for C/T alleles (Sharafi *et al.*, 2012). In this regard, the use of a simple low-cost and high-yielding technique is paramount, since it allows small regional laboratories with limited resources to conduct population genetic studies, thus reducing the sampling bias that may occur in large cosmopolitan cities. This is particularly relevant in regions such as South America, in which the availability of qPCR or sequencing platforms is still limited.

During the last years, there has been a growing interest on the impact of genetic ancestry on the immune response against viral infections (Mersha and Abebe, 2015). The molecular determinants responsible for those associations are being increasingly understood, and interferon pathways and their expression patterns seem to be influenced by genetic ancestry (Miretti and Beck, 2006; Randolph *et al.*, 2021), as suggested by our results.

In the context of the COVID-19 pandemic and considering that IFNλ4 can elicit an antiviral response against RNA viruses, including some coronaviruses, several studies have assessed whether rs12979860 is involved in SARS-CoV-2 susceptibility and COVID-19 outcome. In this regard, it was reported that the T allele was overexpressed in COVID-19 patients compared to the general healthy population (36.2% vs. 26.4%), thus, this allele was proposed as a possible risk factor for COVID-19 (Saponi-Cortes *et al.*, 2021). This was also supported by Rahimi *et al.* (2021), who demonstrated a positive correlation between the survival rate in COVID-19 patients and the rs12979860-CC genotype, which is also favorable to control other infectious diseases caused by RNA viruses. On the other hand, a higher frequency of the CC genotype among COVID-19 patients was reported in a different study, suggesting that people with the C allele (both CT or CC genotypes) are more susceptible to SARS-CoV-2 infection (Agwa *et al.*, 2021). However, only slight differences between infected and control groups are shown (44.7% vs. 44.0%, respectively) and allelic frequencies are the same for both groups (C=34.0%, T=66.0%). In that study, it was also reported that 52.6% of the TT genotypes were classified as severe disease compared to 45.8% and 34.9% in the TC and CC genotypes, respectively (Agwa *et al.*, 2021), which seem to be in line with the results published by Saponi-Cortes *et al.* (2021) and Rahimi *et al.* (2021). It is be noted, also, that the differences shown by Agwa *et al.* (2021) may not be exclusively explained by rs12979860 variants, considering that comorbidities were found in 57.4% of the infected group (and in 18.0% of controls). This highlights the relevance of carrying out a properly designed and unbiased sampling as well as a cautious analysis of the results in order to discern this type of controversies when assessing the differential distribution of these variants in different populations.

## CONCLUSIONS

Given its importance and its apparent association with different infectious diseases, there is a growing interest in assessing IFNλ4 polymorphisms. As a whole, this study describes for the first time the distribution of rs12979860 polymorphism in a healthy sample of the population of Buenos Aires, Argentina, further demonstrating that these frequencies are associated to the composition of the population. This, in addition to being useful in anthropological studies, may contribute to the study of different infectious diseases for which interferon antiviral responses are key.

## ACKNOWLEDGMENTS

We thank Dr. Karina Trono for critical reading of the manuscript. This research was funded by the services provided by AC´s group through STAN-CONICET. Other support came from PIP CONICET 2111.

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BAG | Journal of Basic & Applied Genetics

Vol XXXIII (2): 27-36; December 2022

Journal of the Argentine Society of Genetics

# KNOWLEDGE ABOUT GENETICS AND TRUST IN GENETIC TESTING IN A MID-SIZE CITY IN ARGENTINA

## CONOCIMIENTO SOBRE GENÉTICA Y CONFIANZA EN PRUEBAS GENÉTICAS EN UNA CIUDAD DE TAMAÑO MEDIO EN ARGENTINA

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**Cite this article as:**
Mendoza M., Mazza B., Cabana G.S., Smith L., Di Fabio Rocca F., Delfino H., Martínez C. 2022. KNOWLEDGE ABOUT GENETICS AND TRUST IN GENETIC TESTING IN A MID-SIZE CITY IN ARGENTINA. BAG. Journal of Basic and Applied Genetics XXXIII (2): 27-36.

Received: 05/18/2022
Revised version 1 received: 07/20/2022
Revised version 2 received: 08/02/2022
Accepted: 08/10/2022
General Editor: Elsa Camadro
DOI: 10.35407/bag.2022.33.02.03
ISSN online version: 1852-6233

## ABSTRACT

Public attitudes about genetics appear to depend on the local context. We analyzed survey responses obtained in 2015 from 293 residents of Luján, a city in the province of Buenos Aires, Argentina, who self-assessed their knowledge about genetics and their trust in genetic tests. The survey integrated a larger research project for which consenting adult participants shared demographic and genealogical information and provided saliva samples for genetic ancestry analyses. Participants reported little knowledge but high trust in genetic testing when questioned about knowledge and trust. Well-known media stories of DNA-based forensic genetic investigations to identify the victims of state repression during the military dictatorship may have contributed to the high self-assessment of their genetic knowledge expressed by some participants, regardless of educational attainment. Our analysis provides information that could be used as a baseline to begin unraveling the current level of public trust in genetics in a region of the Global South where genetic testing has become widespread, but people's knowledge of and trust in genetics remain poorly studied.

Key words: genetic tests, knowledge, public attitudes, trust.

## RESUMEN

Las actitudes del público sobre la genética parecen depender del contexto local. Analizamos las respuestas de una encuesta suministrada en 2015 a 293 residentes de Luján, una ciudad de la provincia de Buenos Aires, Argentina, quienes autoevaluaron su conocimiento sobre genética y su confianza en las pruebas genéticas. La encuesta integraba un proyecto de investigación más amplio en el que los adultos participantes que dieron su consentimiento compartieron información demográfica y genealógica y proporcionaron muestras de saliva para un estudio de ancestría genética. Cuando se les preguntó sobre su conocimiento y confianza, los participantes informaron tener poco conocimiento sobre genética, pero mucha confianza en las pruebas genéticas. Historias muy conocidas de los medios de comunicación sobre investigaciones genéticas forenses basadas en el ADN para identificar a las víctimas de la represión estatal durante la dictadura militar pueden haber contribuido a la alta autoevaluación del propio conocimiento genético manifestado por algunos participantes, independientemente de su nivel educativo. Nuestro análisis proporciona información que podría utilizarse como base para comenzar a desentrañar los niveles actuales de confianza pública en la genética en una región del Sur Global donde las pruebas genéticas se han generalizado, pero el conocimiento y confianza de las personas sobre genética están poco estudiados.

Palabras clave: pruebas genéticas, conocimiento, actitudes comunitarias, confianza.

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## INTRODUCTION

Genetic testing is becoming more accessible and widely used everywhere, and researchers are now more interested than before in evaluating community awareness and attitudes about genetics in the public. These studies are often designed to give a better understanding of what factors influence public perspectives on testing and people's reactions to new medical technologies based on genomics (e.g., Bates, 2005; Molster *et al.*, 2009; Bíró *et al.*, 2020; Wang *et al.*, 2021). Studies of attitudes about genetic testing from a public health perspective often aim to understand whether (and under what conditions) receiving information about genes could influence people's health-related behaviors (e.g., Dar-Nimrod *et al.*, 2018; Eum *et al.*, 2018; Peterson *et al.*, 2018; Alvord *et al.*, 2020). Other studies aim to inform the public about privacy regarding healthcare procedures and to assess public opinion on potential outcomes of commercializing technology-based healthcare products (e.g., Horn *et al.*, 2011; Gibbon, 2016; Raz *et al.*, 2020; Gerdes *et al.*, 2021).

Recent literature reviews on knowledge and trust in genetics highlighted that people everywhere generally have positive attitudes about genetic testing that persist even if knowledge about genetics is self-described as low. However, public attitudes could vary according to the technologies and purposes for which genetic knowledge is applied (Condit, 2001, 2010; Etchegary *et al.*, 2009; Chapman *et al.*, 2019; ASHG, 2020; Calabrò *et al.*, 2020).

Earlier research on public attitudes about genetics was conducted with the assumption that attitudes would be stable and unequivocal rather than context-dependent and biased (Condit, 2010). However, more recent cross-sectional studies have uncovered differences in public knowledge and attitudes across multi-year periods (e.g., Henneman *et al.*, 2013). Thus, local sociocultural characteristics and local history could shape individual attitudes over time. Context and local history would have a lasting influence in people's trust in genetics (Cunningham-Burley, 2006; Jonassaint *et al.*, 2010; Canedo *et al.*, 2019).

Researchers have employed various sampling techniques to study public knowledge about genetics and public views on genetic testing, such as convenience sampling (Etchegary *et al.*, 2013; Arafah *et al.*, 2021) and randomized studies (Jallinjoa and Aro, 2000; Haga *et al.*, 2013;LePoire *et al.*, 2019; Wang *et al.*, 2021). Likewise, researchers have employed data collection instruments, such as telephone interviews (Molster *et al.*, 2009), postal surveys (Etchegary *et al.*, 2009), online surveys (Dye *et al.*, 2016; Arafah *et al.*, 2021), focus groups (Bates, 2005; Schumann *et al.*, 2021), Likert-scale questionnaires administered during in-person interviews (Chokoshvili *et al.*, 2017; Kvaratskhelia *et al.*, 2021), or self-administered surveys with fixed-choice and open-ended items (Jonassaint *et al.*, 2010).

Like elsewhere, genetic testing integrated into health-related strategies is increasingly available in Argentina (e.g., Penchaszadeh, 2009, 2013; Vishnopolska *et al.*, 2018). Academic researchers have conducted genetic testing for various ancestry inferences since the 1990s (e.g., Martínez Marignac *et al.*,1999; García and Demarchi, 2006; Corach *et al.*, 2009; Carnese *et al.*, 2011; Avena *et al.*, 2012, 2013). However, genetic ancestry testing to explore individual identities has been far less common (García *et al.*, 2016; Spina *et al.*, 2016; Di Fabio Rocca *et al.*, 2018, 2020). Therefore, local results of scholarly research on genetic ancestry inference were not widely known by the public when we carried out fieldwork for our project.

Our overall project investigated how recent trends in genetic ancestry research in Argentina interacted with the participants' perspectives of national belonging. The study employed a multi-method research design through the generation, analysis, and interpretation of genomic and ethnographic data in a mid-size city in the province of Buenos Aires, Argentina. Over that period, our research team conducted genetic ancestry analyses, ethnographic interviews, and participant observation (Mendoza and Cabana, 2019; Cabana *et al.*, 2022; Mendoza *et al.*, 2022).

This work aims to explore the participants' views and self-assessed levels of knowledge and trust in genetics based on their responses to an in-person survey of adults who consented to participate in our project. The results of our analysis help to begin unraveling public attitudes and trust in genetics among urban populations of Argentina. Additionally, our study provides a preliminary baseline of data to conduct further research on individual's level of knowledge and level of public trust in genetics among other local populations, since this topic remains poorly studied in the country.

## MATERIALS AND METHODS

### *Research site, population sample, and survey*

Our research was carried out in two historic neighborhoods, locally known as El Centro and Santa Elena, in Luján (population: 78,346 inhabitants in 2010), a city with a long colonial history in the province of Buenos Aires, Argentina, now included in the megacity of Buenos Aires (Buzai and Montes Galbán, 2020; Buzai *et al.*, 2021).

The national decennial census divided the city into 87 census tracks. El Centro included 20 census tracks and Santa Elena included three census tracks. We operationalized the number of randomly selected households in those two historic neighborhoods using 2010 census tracks described by Buzai (2014) as social

maps of the city. The minimum number of households per track in El Centro was 142 and the maximum in Santa Elena was 419 (the average number of households per track in the targeted neighborhoods was 274, according to Principi (2021, pers. comm.). The two neighborhoods were characterized by socioeconomic levels varying from very high and high to medium (Principi and Buzai, 2020).

We attempted to recruit one resident per household by leaving recruitment letters at 300 randomly selected households, followed by a personal visit by a research team member. Moreover, the research team made three consecutive attempts to contact a household resident at different times of the day before moving to a different address. We also advertised our project in the local newspaper (Papaleo, 2015), on social media, and in public places, posting large-size announcements in the City Hall, the Public Library, and the local University.

Despite our efforts to engage with residents, many of them did not respond or were reluctant to participate in our project—which included face-to-face contact with team members. Those who declined to engage expressed feelings of lack of security at home, fear of letting anybody in, and overall concern about rising crime levels in the community. For those interested in participating, we offered to meet in public places, but most opted for completing our in-person survey and saliva collection in their homes. A handful of recruitments took place at the local university.

Our field team did not systematically record the census-track locations and verbatim opinions voiced by residents who were not interested in participating in our project. However, we learned some of the reasons expressed by residents who declined to participate during weekly meetings of the entire research team in the winter of 2015. Also, we recorded comments made by participants interested in our project during the initial meeting conducted to explain the informed consent process.

Due to the reasons expressed above, less than one-quarter of the research participants were recruited from the original randomized sample. Following the same criteria and protocol, we fulfilled any remaining openings in our stratified quota by word-of-mouth recruitment of residents of the same neighborhoods whose homes had been previously excluded from spatial randomization. These self-selected participants may have been especially moved by an interest in learning more about our project or by a deeper curiosity about the topic.

Before providing consent to participate, adult residents who expressed interest in our project met with a member of the research team and received a thorough explanation about the topic of genetic ancestry and our research protocols. Consenting residents later met with a member of the research team to answer the face-to-face survey that we analyzed here (they also gave saliva samples that were analyzed elsewhere).

To ensure demographic representation, the sample was stratified a priori into seven age cohorts (from 18 to 71-plus) by asking participants for their chronological ages at the time of recruitment. In addition, we asked for gender identification as an open-ended question and found that participants only declared two categories: "woman" or "man"; we then stratified our sample by these two gender categories. Also, to ensure genomic representation, we did not accept participants who disclosed close biological relationships (i.e., immediate kin or first cousins) with any other enrolled participants.

On the survey form, the following was requested: (a) demographic information (current occupation, educational attainment, birthplace, and length of residence in Luján), (b) family tree information, and (c) responses to two ten-point Likert-scale questions that self-assessed overall knowledge of genetics and level of trust in genetic testing.

We asked: "On a scale of 1 (I know very little) to 10 (I know a lot), what is your level of knowledge of genetics?" and "On a scale of 1 (I trust very little) to 10 (I trust a lot), what is your level of trust in the results of a genetic test?". Thus, participants self-assessed their understanding of genetics choosing from "no knowledge" to "perfect knowledge," and self-assessed their trust in genetic testing choosing from "no trust" to "a great deal of trust" For this analysis, scores were assessed in five intervals: null or very low (1-2); low (3-4); medium (5-6); medium-high (7-8); high (9-10). Additionally, we recorded any pertinent comments offered during survey-taking on the back of the survey form.

In this paper, we analyzed the participants' answers to those two questions and their brief comments. Our final sample consisted of 293 participants (51% women, 49% men, aged 18 years and over, Table 1) residing in the same number of households. Our sample represented 4.6% of all 6,302 households in the targeted neighborhoods.

We calculated the mode, median and relative frequencies of knowledge of genetics and trust in genetic testing. Then, Kendall´s Tau-b test ($t_b$) was applied to explore the degree of association between both variables. Finally, we examined the relationship between gender, age, educational attainment, and occupation with the level of knowledge and trust in genetics through Somers' *d* test, considering the first four demographic variables as explanatory variables (Agresti, 2010). Both statistical tests are based on the number of concordant and discordant pairs of observations. Their values range between -1 and 1, where 1 indicates perfect association independently of the arithmetic sign. Positive associations indicate a higher frequency of concordant than discordant pairs, whereas negative values indicate the inverse. Statistical significance was set at 0.05 using IBM SPSS Statistics for Windows v. 24.0.

**Table 1.** Participants' age, educational attainment, and occupation

| Characteristics | n | % |
|---|---|---|
| *Age Range* | | |
| 18-20 | 8 | 2.7 |
| 21-30 | 66 | 22.6 |
| 31-40 | 53 | 18.2 |
| 41-50 | 29 | 9.9 |
| 51-60 | 57 | 19.5 |
| 61-70 | 49 | 16.8 |
| >70 | 30 | 10.3 |
| *Educational attainment* | | |
| Elementary School incomplete | 4 | 1.4 |
| Elementary School | 16 | 5.5 |
| High School incomplete | 22 | 7.5 |
| High School | 47 | 16.1 |
| 2-year College incomplete | 23 | 7.9 |
| 2-year College | 40 | 13.7 |
| University incomplete | 74 | 25.3 |
| University diploma | 50 | 17.1 |
| Post-graduate incomplete | 8 | 2.7 |
| Post-graduate diploma | 8 | 2.7 |
| *Occupation* | | |
| Employed | 195 | 66.8 |
| Homemaker | 11 | 3.8 |
| Retired | 46 | 15.8 |
| Student | 39 | 13.4 |
| Unemployed | 1 | 0.3 |

## RESULTS

Participants indicated relatively medium knowledge about genetics (Mode= 5; Median= 4.75) and high levels of trust in genetic testing (Mode= 10; Median= 9) (Figure 1). Knowledge about genetics and trust in genetic testing were significantly associated, but the level of association was low ($t_b$= 0.117, $p$= 0.025). Figure 2 shows that the lowest levels of trust in genetic studies correspond with the lowest levels of knowledge about genetics. Still, there is no clear correspondence between the highest levels of both variables. Some participants (n= 41, 14%) self-reported both null or very low knowledge and a great deal of trust in genetic testing.

Educational attainment was the only demographic variable associated with knowledge about genetics, but the association level was low ($d$= 0.139; $p$= 0.008). The participants with incomplete elementary school were the only ones that did not declare a medium-high or high knowledge about genetics. In contrast, a large percentage of the participants with the highest level of education reported to have none to a low level of knowledge (Figure 3). No statistically significant associations were found between participants' level of educational attainment and trust in genetic testing nor with participants' knowledge and trust with gender, age, and occupation.

## DISCUSSION

Studies of trust (interpreted in our survey as *confianza*) often emphasize the optimistic acceptance of a vulnerable situation in which the person who trusts believes the trustee will care for the truster's interest (e.g., Hall *et al.*, 2001). Following Dietz (2011), we considered that trust is based on assessing the other party's trustworthiness. People generally develop their beliefs and assessments of trust in genetics and other matters using social experiences and any technical knowledge they may have (Condit, 2010).

A recent study of trust in genetics distinguished one of two components in the attitudes of the interviewees: (1) the interpersonal relationships of an individual with healthcare professionals and (2) a macro level of trust in institutions or systems (Schumann *et al.*, 2021). Some of the participants' comments recorded in the survey form suggest the presence of these two components in public levels of trust. For example, during survey-taking, some participants made the following comments:

(a) "[Genetics]... is one of the greatest advances ever made."

(b) "There is nothing more credible than genetics."

(c) "[Genetics] ... is one of the few things still reliable in Argentina."

(d) "[I trust it] 99.9 percent."

(e) "[I trust it] with a margin of error."

Most participants self-assessed their level of trust as medium to high. This might appear as an expected outcome, possibly related to individual interest and curiosity about genomics. However, because studies carried out among different populations over time likewise detected high levels of public trust in genetics (Human Genetics Commission, 2001; Ishiyama *et al.*, 2008; Condit, 2010; Henneman *et al.*, 2013; Hishiyama *et al.*, 2019), the medium-to-high levels of trust among

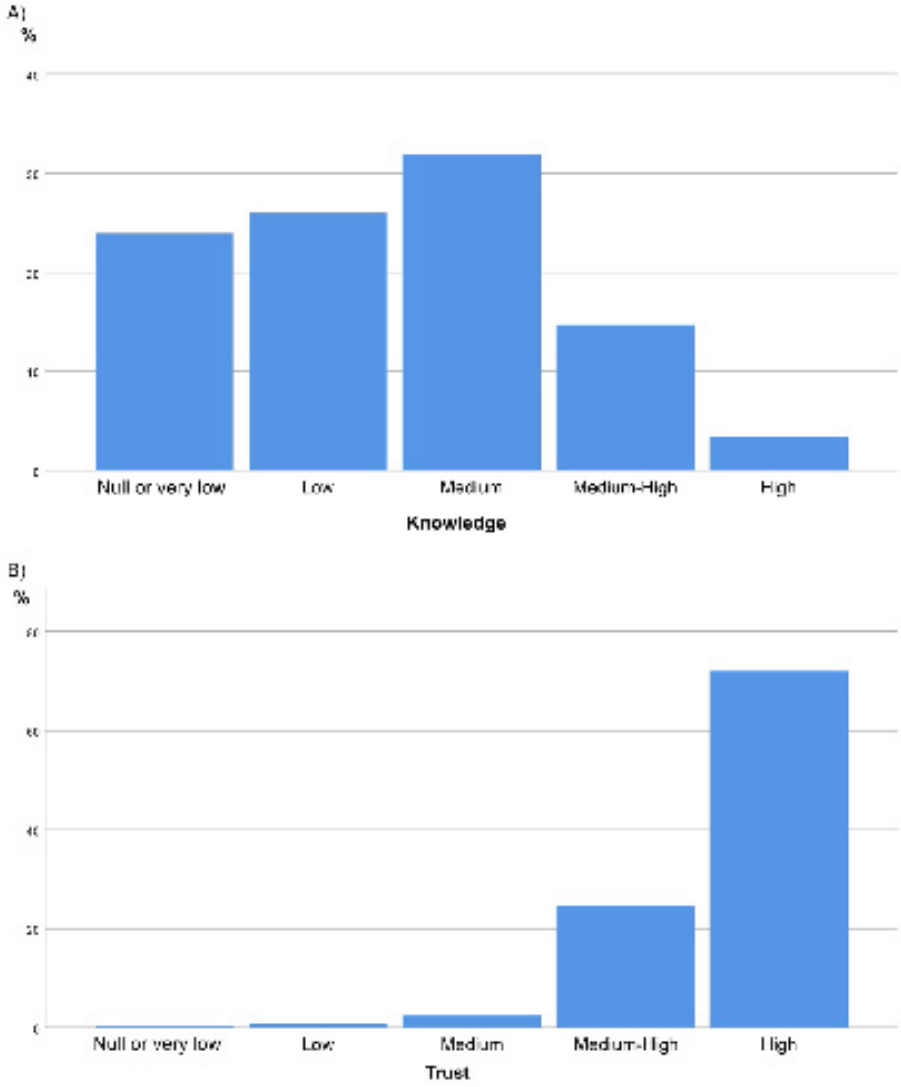


**Figure 1.** Bar chart of score frequencies for participants' knowledge about genetics (A) and trust in genetic testing (B)

participants in our project could be related to additional variables that we do not currently understand.

Some participants in our project expressed what researchers have described as "healthy skepticism" or "selective mistrust" (Schumann *et al.*, 2021) by making, for example, the following comments during survey-taking:

(a) "It depends on the quality of the laboratory and the honesty of the professionals."

(b) "[It depends on] credibility/reputation."

(c) "It depends on what it is used for."

Comparable to attitudes of the public engaged in studies on trust in human genomics in the so-called Global South (de Vries *et al.*, 2014), participants in our project highlighted the importance of knowing whom to trust when explaining their level of trustworthiness in geneticists and laboratories. Many of them, during the initial encounter to discuss the informed consent process, said that they would consent to participate because our project was a collaborative effort between faculty and assistants at the local university and researchers based in universities of the United States. These participants appreciated that our project was not an entirely "foreign" initiative. Thus, people's participation was partially grounded in their trust in local institutions.

Other studies of trust in genetics in different populations over time found that people based their trust on their previous experiences of trusting local institutions (Human Genetics Commission, 2001; Ishiyama *et al.*, 2008; Condit, 2010; Moodley and Singh, 2016; Hishiyama *et al.*, 2019).

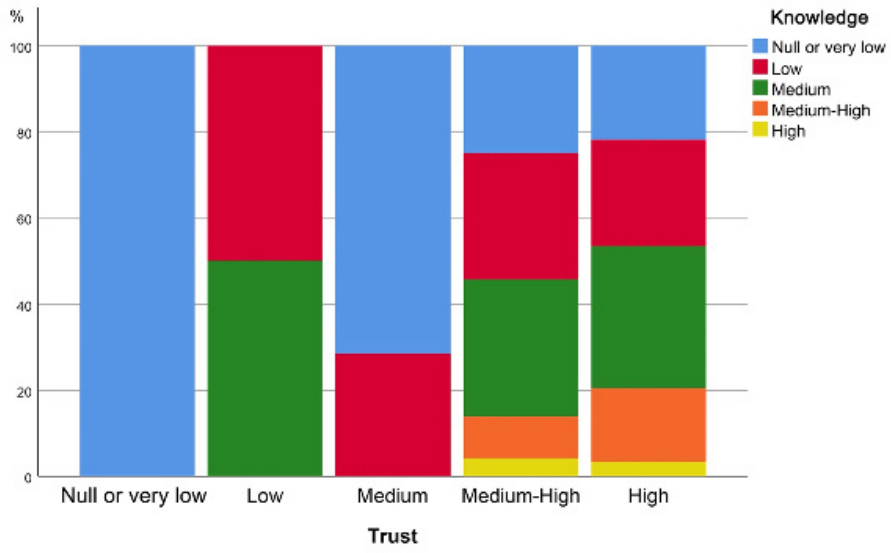


**Figure 2.** Relationship between participants' self-assessed scores for knowledge about genetics and trust in genetic testing

Some residents who declined to participate expressed just the opposite arguments. They said that the foreign component of our collaborative research team, explained in the letter of invitation received by all the randomly selected households, made them feel distrust of our project.

A recent study highlighted the importance of analyzing both mistrust and trust because mistrust points to conditions considered problematic (Schumann *et al.*, 2021). In this work, participants who expressed misgivings referred to their interest in not being subjected to "imperialist" attitudes by research projects funded by scientific institutions in the United States. Residents who declined to participate also raised concerns about sharing personal genetic data with the research team.

The issue of sharing genetic data seems to be very controversial everywhere, and people are hesitant, especially when it comes to sharing genomic data internationally. A review by Majumder *et al.*, (2016) indicated that concerns about misuse of DNA created public distrust and people resisted participating in projects that could potentially misuse or manipulate their genetic material. In the Global South, people recalled instances of "helicopter genetics," describing occurrences of scientists from developed countries "descending" on developing countries to carry out research incompatible with standards of ethics and then using research data without proper credit to local teams and without sharing benefits with the local populations.

Comparable to what was documented among the public of other countries (Majumder *et al.*, 2016; Schumann *et al.*, 2021), people's suspicions in our survey could be interpreted as political statements, articulated critiques of researchers employed by private corporations that profit from accessing local genetics data, or both.

In recent literature reviews of empirical studies, reviewers argued that public understanding of genetic testing evolves over the years, and populations in different countries often hold particular views about genomics due to variable exposure to information about genetics and differences in their public health systems (e.g., Henneman *et al.*, 2013; Chokoshvili *et al.*, 2017; Kvaratskhelia *et al.*, 2021).

The participants in our study said (usually during the initial meeting to discuss the informed consent) that they learned Mendelian genetics from elementary through high school and expanded their understanding of molecular genetics as they advanced in their education. In the two urban neighborhoods of Luján, people said that they usually accessed information about genomics through TV programs, the Internet, print, and social media. Generally, people were familiar with concepts such as genes and DNA and understood that parents pass hereditary material to their children.

Rather than utilizing linear models of transmission of information to interpret the process of receiving and processing information -as was assumed by previous research (e.g., Michael and Carter, 2001; Petersen, 2001; Levitt, 2003)- the participants in those urban

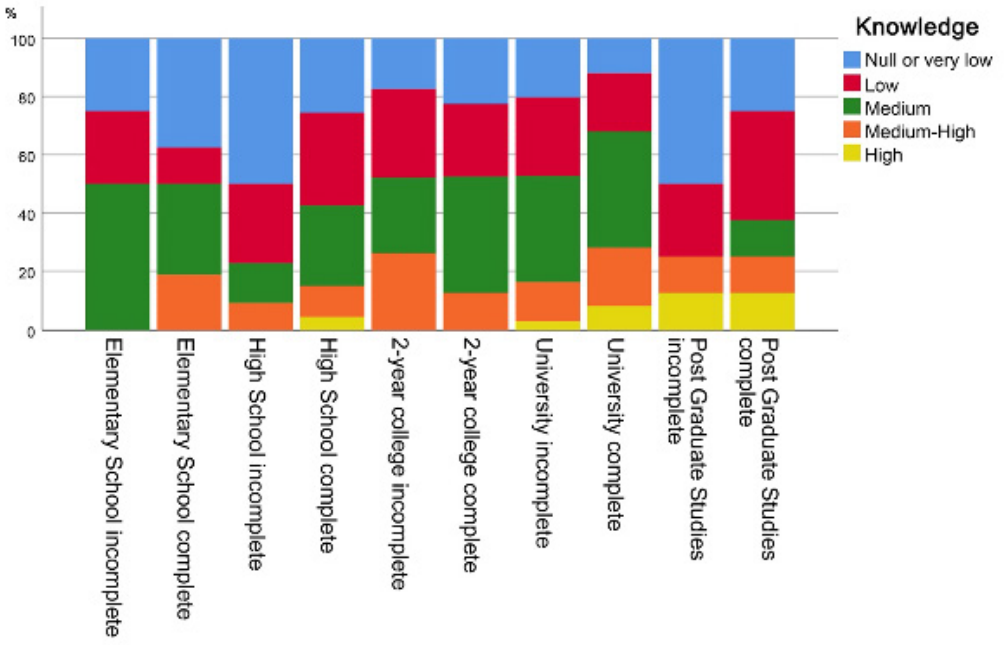


**Figure 3.** Relationship between participants' self-assessed score for knowledge about genetics and participants' educational attainment

neighborhoods in Argentina, like elsewhere, appear to utilize complex and critical approaches to handle scientific information, not directly related to formal schooling. As argued by Bates (2005), formal schooling would only inform part of the public's understanding of recent advances in genetic technology. Typically, people would form their ideas by critically dealing with messages about genetics seen in news media, popular television, documentaries, and science-fiction films. Thus, to a large extent, popular culture, more than formal education, would shape people's understanding of genetics.

Elaborating on participant's knowledge of genetics during the initial meeting to discuss the process of informed consent, several of them mentioned their awareness of ongoing forensic anthropology investigations to identify the victims of state repression and the children of missing persons (*desaparecidos*) during Argentina's military dictatorship (1976-1983) (Jelin, 2009; Penchaszadeh, 2011; Guglielmucci, 2013; Kling *et al.*, 2017; Lerman, 2017). Widespread public awareness of DNA-based forensic genetics research in Argentina could have contributed to the participants' self-reported knowledge/understanding of genetics, regardless of their educational attainment. Also, well-known media stories of DNA-based forensic genetic identification may have contributed to the participants' self-assessed high level of trust.

Overall, researchers in other countries have found no clear statistical patterns connecting people's level of genetic knowledge and their attitudes toward genetics. Research and literature reviews suggest that the effects of education could be contradictory (Condit, 2010; Etchegary, 2014; Chapman *et al.*, 2019). Nonetheless, education continues to be a pertinent demographic variable in field studies about knowledge and trust in genetics. In our survey, educational attainment was associated with knowledge, but the association level was low. Some researchers found that greater knowledge about genetics was correlated with the level of education and associated with trust in the benefits of genetic testing, but other studies pointed to the opposite (Bíró *et al.*, 2020).

Highly educated people with considerable knowledge about science would sometimes express *more* criticism and be *less* trusting about genomic developments than individuals with lower levels of education (Jallinjoa and Aro, 2000). People in the so-called Global North have expressed skepticism about genetic tests, a development that Schumann *et al.* (2021) attribute to the decline of trust in authorities, experts, and institutions. The rather impressive level of self-assessed trust in genetics in our survey could instead point to the optimistic acceptance of science identified by Hall *et al.* (2001).

A limitation of our analysis is that with the two questions in our survey we assessed people's knowledge

of and trust in genetics only among those urban residents who consented to participate in our project. Further research is needed to examine responses by other residents in larger randomized samples residing in neighborhoods with different socioeconomic levels, and in rural locations. We did not systematically record the census-track location and verbatim opinions voiced by residents who declined to participate, but we learned about the reasons expressed by people who were not interested in our project during regular updates by members of our research team. Moreover, although our initial intention was to work with a probabilistic sample, the analyzed sample is not probabilistic. Thus, our inferential analysis of the results must be taken with caution.

## CONCLUSION

Studying the levels of trust (interpreted as *confianza*) that people place on genetics and learning how they self-assessed their knowledge (interpreted as *conocimiento*) about genetics are important because shifting individual perspectives may influence people's willingness to participate in research projects that incorporate genetic testing. The 293 responses to the two ten-point Likert-scale questions self-assessing the overall knowledge of genetics and the level of trust in genetic testing in Argentina, could offer a preliminary baseline to start developing new research paths for future studies on the topic. Our analysis leads us to conclude that paying attention to issues of trust and mistrust in the community could facilitate and improve the process of obtaining an ethically sound and socially acceptable informed consent for research projects.

## ACKNOWLEDGMENTS

This research has been evaluated and funded by the U.S. National Science Foundation. It has been evaluated and approved by the Institutional Review Boards (IRB) at the University of Tennessee, the University of Oregon, and the University of New Mexico, as well as by the Comité de Ética de la Región Sanitaria VII Hospital de Agudos "Dr. Ramón Carrillo." The anonymized data analyzed here were collected during a research project in Argentina entitled "A longitudinal study of the role of expert knowledge in the interpretation and reception of genetic information"; National Science Foundation, Senior Research Grant Award No. SES-1354185, period 2014-2019. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all participants for being included in the study.

We are grateful for the expert advice provided by Drs. Gustavo Buzai and Noelia Principi, Laboratorio de Análisis Espacial y Sistemas de Información Geográfica, Universidad Nacional de Luján. A previous version of this paper benefited from generous feedback by the participants in a Research Brief organized on February 23, 2021, by Global and International Studies, Western Michigan University.

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BAG | Journal of Basic & Applied Genetics

Vol XXXIII (2): 37-44; December 2022

Journal of the Argentine Society of Genetics

# CLONAL DETECTION OF *Streptococcus agalactiae* Lehmann AND Neumann PARENTAL STRAINS BY RANDOM AMPLIFICATION OF POLYMORPHIC DNA

# DETECCIÓN CLONAL DE CEPAS PARENTALES DE *Streptococcus agalactiae* Lehmann y Neumann POR AMPLIFICACIÓN ALEATORIA DE ADN POLIMÓRFICO

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**Cite this article as:**
Cortese, I.J., Novosak M.G., Oviedo P.N., Cannistraci Giolito R.E., Laczeski M.E. 2022. CLONAL DETECTION OF *Streptococcus agalactiae* Lehmann y Neumann PARENTAL STRAINS BY RANDOM AMPLIFICATION OF POLYMORPHIC DNA. BAG. Journal of Basic and Applied Genetics XXXIII (2): 37-44.

Received: 03/29/2021
Revised version received: 07/13/2021
Accepted: 09/22/2021
General Editor: Elsa Camadro
DOI: 10.35407/bag.2022.33.02.04
ISSN online version: 1852-6233

Available online at
www.sag.org.ar/jbag

## ABSTRACT

*Streptococcus agalactiae* (GBS) causes invasive infections in newborns, being the most frequent the maternal transmission. Epidemiological studies use molecular techniques that assess genetic diversity, including random amplification of polymorphic DNA (RAPD) that is found to be accessible, sensitive and uses arbitrary primers to amplify polymorphic segments of DNA by PCR. The objective was to determine the clonal relationship between GBS strains recovered from mothers and their respective newborns. Four pairs of GBS isolates obtained from vaginal-rectal swabs of mothers and blood cultures of their newborns were studied with RAPD. Primers OPS11, OPB17 and OPB18 were used to select one with the ability to discriminate between non-genetically related strains. The Hunter-Gaston formula that establishes the discrimination index (D) was used; when D>0.90, it is considered that the isolates belong to different clones. The amplification profiles for the eight isolates, using each primer independently, allowed to calculate a D=1 for OPS11, and D=0.84 for OPB17 and OPB18. Therefore, OPS11 was selected for the study of the clonal relationship of the isolates, and similar amplification profiles were found by RAPD for each mother-newborn pair of GBS isolates. Different amplification profiles were observed between pairs of mother-newborn strains, which reveals the discrimination between unrelated strains, confirmed by pulsating field electrophoresis (PFGE). These results indicated vertical transmission for each studied case and robustness of the OPS11 primer. Appropriate conditions of the RAPD trial were found, which is useful for epidemiological studies.

Key words: *Streptococcus agalactiae*, neonatal disease, molecular epidemiology, RAPD technique, vertical transmission

## RESUMEN

*Streptococcus agalactiae* (SGB) produce infecciones invasivas en neonatos siendo la transmisión materna la más frecuente. Estudios epidemiológicos utilizan técnicas moleculares que evalúan la diversidad genética, entre ellas la de amplificación aleatoria de ADN polimórfico (RAPD) que resulta ser accesible, sensible y utiliza cebadores arbitrarios para amplificar segmentos polimórficos de ADN mediante PCR. El objetivo fue determinar la relación clonal entre aislamientos de SGB recuperados de madres y sus respectivos recién nacidos. Se estudiaron por RAPD cuatro parejas de aislamientos de SGB obtenidos de hisopados vagino-rectales de madres y de hemocultivos de sus neonatos. Se utilizaron los cebadores OPS11, OPB17 y OPB18 para seleccionar uno con capacidad de discriminar entre cepas no relacionadas genéticamente. Se utilizó la fórmula de Hunter-Gaston que establece el índice de discriminación (D), cuando D>0,90 se considera que los aislamientos pertenecen a clones diferentes. Los perfiles de amplificación para los ocho aislamientos, empleando independientemente cada cebador, permitieron calcular un D=1 para OPS11, y D=0,84 para OPB17 y OPB18. Por lo tanto, OPS11 fue seleccionado para el estudio de la relación clonal de los aislamientos, encontrándose perfiles de amplificación similares por RAPD para cada par de cepas madre-recién nacido. Se observaron diferentes perfiles de amplificación entre los pares de cepas madre-recién nacido, lo que revela la discriminación entre cepas no relacionadas, resultados confirmados por electroforesis en campo pulsante (PFGE). Estos resultados indican transmisión vertical para cada caso estudiado y robustez del cebador OPS11. Se encontraron condiciones apropiadas del ensayo de RAPD, lo que es útil para estudios epidemiológicos.

Palabras clave: *Streptococcus agalactiae*, enfermedad neonatal, epidemiología molecular, técnica RAPD, transmisión vertical.

## INTRODUCCIÓN

*Streptococcus agalactiae* (*Streptococcus* del grupo B, SGB) es un microorganismo que coloniza el tracto gastrointestinal y genitourinario humano (Barcaite *et al.*, 2014). Su colonización puede ser transitoria, intermitente o crónica, y los factores que influyen en su persistencia aún son desconocidos (Patras *et al.*, 2015; Sarrión-Sos *et al.*, 2018).

La enfermedad invasiva por SGB se identificó por primera vez en adultos y neonatos en la década de 1960 (Raabe y Shane, 2019). La vía de transmisión al feto o al recién nacido ocurre a partir de la colonización recto-vaginal materna (Russell *et al.*, 2017). En mujeres embarazadas colonizadas, las bacterias pueden ascender desde el tracto genitourinario hacia el útero o la vejiga. En el útero, pueden afectar las membranas fetales y causar corioamnionitis, lo que puede provocar parto prematuro, aborto espontáneo o la muerte fetal intrauterina. Alternativamente, la bacteria puede invadir el tracto respiratorio del recién nacido mediante la aspiración del líquido amniótico o por su pasaje por el canal de parto y causar neumonía. También puede destruir el revestimiento alveolar utilizando factores de virulencia y llegar al torrente sanguíneo, desencadenando bacteriemia y sepsis. Una vez en sangre, las bacterias pueden cruzar la barrera hematoencefálica y migrar al líquido cefalorraquídeo (LCR) produciendo meningitis (Hanna y Noor, 2020). Las tasas de transmisión del SGB madre-recién nacido varían en todo el mundo, sin embargo, los informes describen tasas de alrededor de 0,53 por 1.000 nacidos vivos (Do Nascimento *et al.*, 2019).

Se describieron dos formas de enfermedad invasiva por SGB en niños en función de la edad de presentación: la enfermedad de inicio temprano (*early onset disease*; EOD) que ocurre durante los primeros seis días de vida, y la enfermedad de inicio tardío (*late onset disease*; LOD) que se desarrolla entre los siete y 90 días después del nacimiento, se manifiesta con meningitis y bacteriemia y es de transmisión materna o intranosocomial (Manrique Martín *et al.*, 2020).

La EOD, la más severa y de mayor importancia clínica, surge de la transmisión vertical de una madre colonizada a su recién nacido durante o inmediatamente antes del nacimiento, con signos clínicos que ocurren dentro de las 24-48 h en más del 90% de los casos (Collin *et al.*, 2019). Los factores de riesgo para la EOD incluyen colonización vaginal o rectal materna, bacteriuria por SGB durante el embarazo, trabajo de parto prolongado, rotura prematura de membranas, bajo peso al nacer, prematuridad, fiebre intraparto y enfermedad sistémica materna por SGB (Nanayakkara *et al.*, 2018; Raabe y Shane, 2019). Por otro lado, las manifestaciones típicas de la EOD incluyen dificultad respiratoria como apnea o taquipnea, respiración entrecortada, cianosis, letargo, mala alimentación, distensión abdominal, palidez, ictericia, taquicardia e hipotensión. La bacteriemia es la forma más común de EOD producida por SGB, manifestada en el 80% de los casos, mientras que la neumonía y meningitis son menos comunes y representan el 15% y 5% a 10%, respectivamente (Raabe y Shane, 2019; Hanna y Noor, 2020).

La gravedad de la EOD está determinada en gran medida por factores de virulencia necesarios para la interacción célula huésped-bacteria (Herbert *et al.*, 2004); uno de ellos, la cápsula polisacárida, permite la clasificación de SGB en los serotipos Ia, Ib, II a IX (Hanna y Noor, 2020). Algunos serotipos se asocian con clones más virulentos y, por lo tanto, con una propensión a la enfermedad invasiva por SGB, por ejemplo, el III, que se asocia con frecuencia con el complejo clonal hipervirulento y representa el 43% de la EOD y el 73% de la LOD, y el IV, que causa el 97% de las infecciones severas en neonatos (Raabe y Shane, 2019; Russell *et al.*, 2017). A partir de 1996 el Centro para el Control y la Prevención de Enfermedades (CDC, 1996; CDC, 2002; CDC, 2010) publicó directrices para la prevención de la enfermedad perinatal, a partir de la búsqueda de SGB en toda mujer embarazada entre 35 y 37 semanas de gestación. Teniendo en cuenta la importancia de implementar acciones y decisiones de salud pública, en nuestro país, desde abril del año 2008, se encuentra vigente la Ley Nacional Nº 26.369 (2008) que establece la obligatoriedad de la búsqueda de portación de SGB en toda mujer con edad gestacional entre 35 y 37 semanas, presenten o no condiciones de riesgo.

En las últimas tres décadas se han desarrollado técnicas moleculares orientadas al estudio de la diversidad genética entre microorganismos estrechamente relacionados, entre ellas polimorfismos de longitud de fragmentos de restricción (RFLP) (Chatellier *et al.*, 1996), electroforesis en gel de campo pulsado (PFGE) (Manning, 2003), electroforesis enzimática multilocus (Quentin *et al.*, 1995), ribotipado (Huet *et al.*, 1993) y amplificación aleatoria de ADN polimórfico (RAPD) (Toresani *et al.*, 2001). Si bien se ha demostrado que PFGE es una técnica precisa y fiable, utilizada como método estándar en laboratorios de mediana y alta complejidad (Åberg *et al.*, 2019), su aplicación es compleja, costosa y requiere de mucho tiempo en comparación con la facilidad y velocidad de ejecución de las técnicas basadas en la reacción en cadena de la polimerasa (PCR). En este sentido la técnica RAPD es accesible y sensible, por lo que su aplicación en estudios de variabilidad es especialmente útil para realizar análisis intraespecíficos. Esta técnica se realiza con pequeñas cantidades de ADN y con cebadores sintéticos cortos con una secuencia al azar de aproximadamente 9-10 bases de longitud, cuya selección es un paso

crítico. Estos marcadores tienen la ventaja de amplificar regiones del genoma que pueden ser transcriptas como así también regiones no codificantes (Rolim *et al.*, 2011). La amplificación de fragmentos se produce debido a las diferencias en la distancia entre los sitios de unión del cebador produciendo perfiles de bandas específicos para una cepa determinada (Idil y Bilkay, 2014).

En este contexto, si bien la pesquisa de colonización materna y la instauración de la profilaxis intraparto (PIP) con antimicrobianos a toda embarazada colonizada es obligatoria, la mortalidad del recién nacido es aún elevada, principalmente en partos pre-término o con factores de riesgo subyacentes. Por ello, el desafío actual está orientado a desarrollar nuevos métodos de diagnóstico y nuevas estrategias de prevención que incluyan el desarrollo de vacunas a fin de prevenir la incidencia de este microorganismo en el recién nacido (Chen *et al.*, 2013; Szymusik *et al.*, 2014).

Además, resulta importante para la región y el país, realizar estudios epidemiológicos ligados a la portación materna de SGB y su transmisión al recién nacido, y a la posible transmisión horizontal en centros de salud. Por ello, el objetivo de esta investigación fue determinar la relación clonal entre aislamientos de SGB recuperados de madres colonizadas y sus respectivos recién nacidos de dos hospitales argentinos por la técnica RAPD.

## MATERIALES Y MÉTODOS

### *Bacterias*

Se trabajó con cuatro aislamientos de SGB recuperados de mujeres luego del parto (estas pacientes habían llegado a los centros de salud sin realización previa de búsqueda de portación materna de SGB durante el embarazo) y cuatro aislamientos recuperados de sus respectivos recién nacidos con enfermedad invasiva por SGB, entre los años 2011 y 2016. Tres pares de aislamientos madre-hijo fueron del Hospital Escuela de Agudos "Dr. Ramón Madariaga" de Posadas, Misiones y el par restante del Hospital Materno Provincial "Dr. Raul F. Lucini" de Córdoba, Córdoba. Los seis aislamientos de Posadas se identificaron como E4669 madre y O4663 recién nacido; E2949 madre y O2963 recién nacido; E6149 madre y O6150 recién nacido (además, para la técnica de PCR se incluyó el aislamiento O6151 recién nacido recuperado de LCR del mismo paciente) y los dos de Córdoba como 6 madre y 7 recién nacido.

Las muestras maternas se tomaron con hisopo estéril del tercio anterior de vagina y de la zona ano-rectal. Se conservaron en medio de transporte Stuart (Venturi Transystem Stuart, Copan, Italia S.p.A) hasta la siembra en 1-2 mL de caldo Todd-Hewitt suplementado con colistina (10 µg $mL^{-1}$, Britania, Argentina) y ácido nalidíxico (15 µg $mL^{-1}$, Britania, Argentina) y se incubaron a 37° C durante 24 h. Luego se sembraron con técnica para aislamiento en placas de agar con sangre ovina al 5% (Rafaela, Argentina). Las placas se incubaron en atmósfera microaeróbica a 37° C durante 24 h. La identificación bacteriana se realizó mediante pruebas bioquímicas convencionales y serología de grupo (aglutinación con partículas de látex, Phadebact Strep B Test-ETC International-Bactus AB, Suecia). El serotipo se determinó por técnica de aglutinación Statens Serum Institute (Strep-B. Latex, Copenhague, Dinamarca). Ambas técnicas se realizaron siguiendo las recomendaciones del fabricante.

De los recién nacidos se tomaron muestras de hemocultivos y LCR siguiendo las recomendaciones internacionales. La siembra e identificación bioquímica de los aislamientos neonatales de SGB se realizó con las mismas técnicas utilizadas para los aislamientos maternos. En la Tabla 1 se detallan las características de los aislamientos de SGB recuperados de los recién nacidos.

Todos los aislamientos se conservaron en leche descremada al 20% a -80° C en el Cepario de la Cátedra de Bacteriología, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, hasta ser utilizados en esta investigación.

### *Extracción de ADN*

Los aislamientos de SGB se cultivaron en caldo Todd-Hewitt (Britania, Argentina) y se incubaron a 37° C durante 24 h. La extracción de ADN bacteriano se realizó con el protocolo de Sambrook modificado (Cariaga Martinez y Zapata, 2007).

### *Selección de cebadores*

Se realizó un análisis comparativo de los patrones de bandas generados para los aislamientos a partir de la técnica RAPD utilizando tres cebadores previamente diseñados para SGB (Martinez *et al.*, 2000) (Tabla 2). Se evaluó la utilidad de cada cebador para detectar aislamientos genéticamente no relacionados con el Índice de Simpson (D), calculado a partir de la siguiente fórmula (Hunter y Gaston, 1998).

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j \, (n_j - 1)$$

Donde N es el número total de los aislamientos en la población de la muestra, S es el número total de patrones de RAPD obtenidos y nj es el número de aislamientos pertenecientes al tipo j-ésimo. Un índice superior a 0,90 permite la interpretación de resultados de tipificación fiables (Hunter y Gaston, 1998; Martinez *et al.*, 2000; Shadbad *et al.*, 2020).

**Tabla 1.** Datos de los aislamientos de *Streptococcus agalactiae* recuperados de recién nacidos con enfermedad invasiva de inicio temprano. Posadas-Córdoba, 2011-2016.

| Código del aislamiento | Modo de nacimiento | Origen | Edad (días) | Sitio de aislamiento de SGB | Fecha de muestra |
|---|---|---|---|---|---|
| 7 | Parto | Córdoba | 2 | LCR* | 03/03/2014 |
| 06150** | Parto | Posadas | 1 | Hemocultivos | 16/03/2011 |
| 06151** | Parto | Posadas | 1 | LCR | 16/03/2011 |
| 02963 | Parto | Posadas | 2 | Hemocultivos | 05/06/2013 |
| 04663 | Parto | Posadas | 1 | Hemocultivos | 14/04/2016 |

*LCR (líquido cefalorraquídeo) **Aislamientos recuperados del mismo paciente

**Tabla 2.** Secuencias nucleotídicas de los cebadores utilizados para la técnica RAPD.

| Nombre del cebador | Secuencia del cebador 5´ - 3´ | Referencia bibliográfica |
|---|---|---|
| OPS11 | AGTCGGGTGG | Martinez *et al*., 2000 |
| OPB17 | AGGGAACGAG | Martinez *et al*., 2000 |
| OPB18 | CCACAGCAGT | Martinez *et al*., 2000 |

### *Generación de patrones RAPD*

La reacción de amplificación se realizó en un volumen final de 50 µL compuesto por solución tampón para PCR 1X (10X: 100 mM Tris-HCl pH 9, 500 mM KCl, 1% Triton® X-100), 100 mM dNTPs, 0,4 µM de cebador (Tabla 2), 2,5 mM $MgCl_2$, 50 ng $\mu L^{-1}$ de ADN molde y 2,5 U de ADN polimerasa Taq (Inbio Highway, Argentina).

Se utilizó un solo cebador en cada reacción de PCR. Las amplificaciones se realizaron en un termociclador Multigene TM II (Labnet internacional Inc., EE. UU.). El perfil de ciclado consistió en: pre-desnaturalización a 94° C durante 5 min, 40 ciclos de amplificación (desnaturalización a 94° C durante 1 min, hibridación a 36° C durante 1 min, extensión a 72° C durante 1 min) y extensión a 72° C durante 5 min. Los productos de amplificación se revelaron en gel de agarosa al 1,2% (p/v) teñido con GelRed® (Sigma-Aldrich, Alemania). La electroforesis se realizó en una cuba electroforética (Subsistema de electroforesis 70 Labnet International) a 100 V durante 3 h y las bandas se visualizaron usando un transiluminador UV (Modelo MUV 21-312-220).

### *Análisis de patrones de bandas obtenidos por RAPD y construcción del Dendrograma*

En base a la distancia de migración, el tamaño de las diferentes bandas fue medido y extrapolado al correspondiente marcador de peso molecular mediante el programa *Graphpad Prism*.

Los dendrogramas se generaron con el programa estadístico para Excel, *XLSTAT* utilizando el método de grupos de pares no ponderados con media aritmética (UPGMA). Se consideró que los aislamientos que tenían una similitud >75% en el índice de Dice con una

tolerancia de 3,5 tenían el mismo origen, lo que indicaría una posible transmisión entre la madre y el recién nacido.

### *Electroforesis en gel de campo pulsante*

Los pares de cepas madre-recién nacido fueron enviadas al Servicio de Antimicrobianos de la Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) "Dr. Carlos Malbrán" para evaluar su relación clonal mediante PFGE. De acuerdo con el informe recibido, el ADN total se digirió con las enzimas de restricción *SmaI* y *ApaI* y se trabajó de acuerdo con el protocolo propuesto por Faccone *et al.* (2010) utilizando un equipo CHEF-DRIII.

## RESULTADOS

Se detectó el serotipo III en tres pares de aislamientos madre-recién nacido, y el serotipo Ia en uno de ellos.

El cebador OPS11 mostró un perfil de amplificación nítido y un valor D igual a 1, mientras que los cebadores OPB17 y OPB18 mostraron patrones de bandas no concluyentes y un valor D de 0,84. En la Figura 1 se muestran los perfiles de bandas obtenidos a partir de la técnica RAPD utilizando los tres cebadores. En base a los patrones de bandas observados y a los valores obtenidos para D, se seleccionó el cebador OPS11 para el análisis de los pares de cepas madre-recién nacido, y para su posterior comparación con los resultados generados por la técnica PFGE.

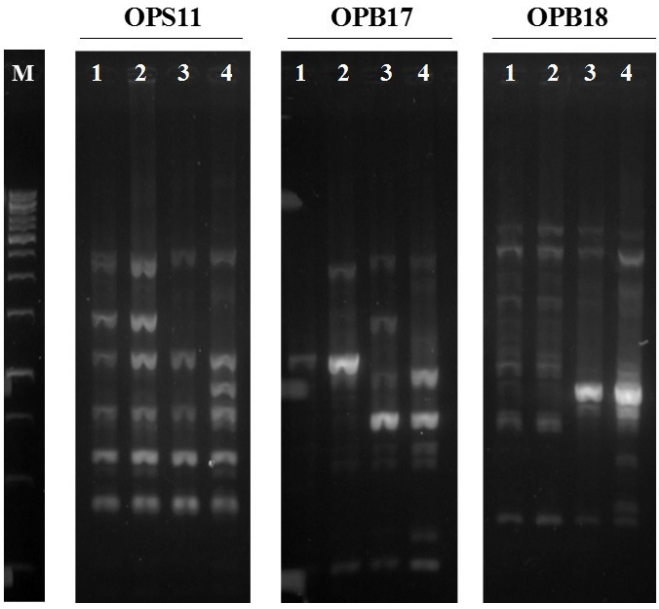


**Figura 1.** Perfiles de bandas electroforéticas obtenidos por la técnica RAPD utilizando los cebadores OPS11, OPB17 y OPB18.

El cebador OPS11 permitió caracterizar individualmente a cada cepa estudiada. El análisis genómico de los aislados de SGB reveló cuatro perfiles de bandas diferentes indicando la presencia de cuatro clones, lo que demuestra una relación entre el aislamiento materno y la infección del recién nacido en los cuatro casos. Por otro lado, no se evidenció una relación epidemiológica entre las cepas de diferentes pares de madre-recién nacido, a pesar de pertenecer al mismo serotipo (Figura 2).

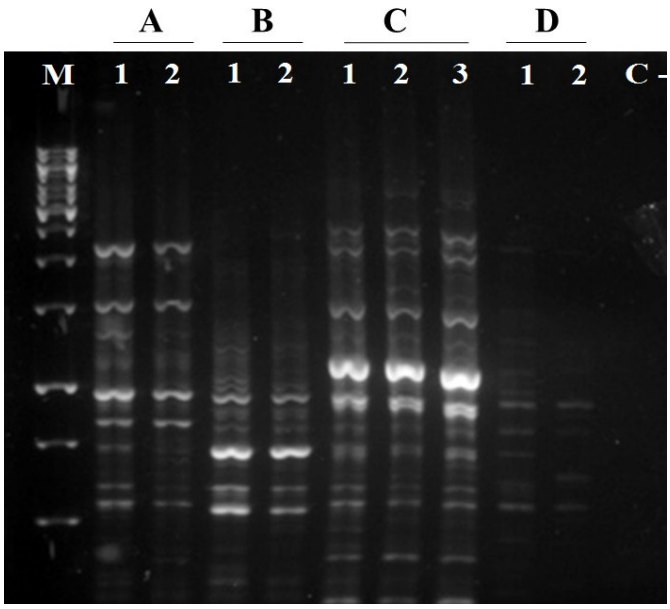


**Figura 2.** Perfiles de bandas electroforéticas obtenidos por la técnica RAPD utilizando el cebador OPS11, comparación entre los aislamientos de *Streptococcus agalactiae* obtenidos de madre-recién nacido: (A) E4669 madre y O4663 recién nacido; (B) E2949 madre y O2963 recién nacido; (C) E6149 madre y O6150 recién nacido; O6151 recién nacido y (D) 6 madre y 7 recién nacido.

El informe de PFGE del Servicio de Antimicrobianos de la Administración Nacional de Laboratorios y Servicios de Salud (ANLIS) "Dr. Carlos Malbrán", fue el siguiente: La restricción con *ApaI*, sólo permitió tipificar las cepas 6 madre y 7 recién nacido que dieron perfiles indistinguibles entre sí. Los seis aislamientos restantes resultaron no con *ApaI* en tres oportunidades. Usando *SmaI*, los aislamientos 6 madre y 7 recién nacido presentaron un perfil de restricción similar con dos bandas de diferencia, lo que implicaría que corresponden a subtipos de un mismo clon. Los seis aislamientos restantes E4669 madre y O4663 recién nacido; E2949 madre y O2963 recién nacido; E6149 madre y O6150 recién nacido, que no pudieron tipificarse con *ApaI*, al hacer la restricción con *SmaI* se pudieron diferenciar en tres tipos clonales. Cada uno de estos tipos clonales corresponde a una dupla madre e hijo, indistinguibles entre sí.

Los resultados obtenidos por PFGE permiten determinar que en todos los casos los pares de aislamientos madre-recién nacido estuvieron genéticamente relacionados entre sí, respaldando los resultados generados previamente con la técnica RAPD utilizando el cebador OPS11.

## DISCUSIÓN

La diversidad genética entre cepas de SGB estrechamente relacionadas ha sido estudiada a partir de diferentes técnicas moleculares. Algunas metodologías como PFGE implican pasos que consumen mucho tiempo, reactivos costosos y equipos sofisticados, por lo que está restringida a laboratorios de referencia donde es aplicada como técnica estándar (Hansen *et al.*, 2004). Sin embargo la técnica RAPD, entre otras, ha sido utilizada en los últimos 30 años para determinar la relación entre los aislamientos de SGB (Shadbad *et al.*, 2020; Taniyama *et al.*, 2020). Se ha aplicado ampliamente para el análisis epidemiológico y se demostró su utilidad para el análisis genético rápido de cepas de SGB siendo capaz de detectar heterogeneidad genómica dentro de serotipos específicos (Brandolini *et al.*, 2014).

Estudios similares a nuestro trabajo fueron realizados por Brandolini *et al.* (2014) sobre casos de transmisión de SGB a través de la ingestión de leche materna. Para su investigación utilizaron la técnica RAPD para el análisis genético de hemocultivos, muestras de LCR y de leche materna. Los resultados revelaron cuatro perfiles de bandas diferentes que indicaron la presencia de cuatro clones, lo que demostró una relación entre la leche materna y la infección de los lactantes en los cuatro casos. Además, observaron que los patrones de bandas de SGB fueron idénticos dentro de cada conjunto de cepas, es decir, entre los aislamientos de hemocultivo y LCR neonatal y de la leche materna, lo que indicó la presencia del mismo clon. Por otro lado, los aislamientos obtenidos de cada par madre-recién nacido mostraron patrones de bandas claramente diferentes, descartando una correlación epidemiológica al igual que en el presente trabajo. De manera similar, Nanayakkara *et al.*, (2018), analizaron aislamientos obtenidos de madre-recién nacido mediante la técnica RAPD, el análisis reveló la presencia de una misma cepa de SGB en el 2,5% de los casos, indicando una transferencia entre la madre y el recién nacido.

Las investigaciones realizadas por Nanayakkara *et al.*, (2018) y Brandolini *et al.* (2014) apoyan los resultados obtenidos en el presente trabajo donde se detectaron cuatro patrones de bandas diferentes correspondientes a los cuatro pares de aislamientos madre-recién nacido analizadas. Este resultado apoya la idea que la colonización por SGB del recién nacido durante su paso por el canal de parto es la principal causa de EOD.

Por otra parte, un estudio realizado con PFGE por Hansen *et al.*, (2004) en aislamientos de SGB demostró que la cepa colonizadora era homogénea y estable en cada mujer embarazada estudiada. Los resultados indicaron que prácticamente todas las mujeres fueron colonizadas por un solo clon de SGB y en solo unas pocas, se encontraron dos clones diferentes simultáneamente. Para la aplicación de PFGE utilizaron las enzimas de restricción *ApaI*, *XhoI* y *NotI*, que no arrojaron resultados satisfactorios, y las enzimas *SmaI* o *SalI*, con las que se obtuvieron patrones de bandas de buena calidad. De la misma manera, en el presente trabajo la enzima de restricción *ApaI* solo logró distinguir uno de los pares de clones, mientras que *SmaI* permitió diferenciar cuatro tipos clonales correspondientes a cada par de cepas madre-recién nacido. Los resultados obtenidos por Hansen *et al.*, (2004) refuerzan la idea de que los recién nacidos se infectan con el mismo clon que coloniza a sus madres, a partir de la transmisión vertical de SGB.

De manera similar Hirai *et al.*, (2020) aplicaron la técnica PFGE para la caracterización de cepas invasivas de SGB aisladas de recién nacidos y adultos que desarrollaron la enfermedad. Para ello, al igual que en el presente trabajo, utilizaron la enzima de restricción *SmaI*. A partir de los patrones de bandas generados identificaron clones de SGB asociados a iguales serotipos, genotipos de resistencia y susceptibilidad a antibióticos, concluyendo la existencia de una transmisión horizontal en la comunidad durante 10 años.

El presente trabajo concuerda con otros investigadores en que RAPD es una técnica rápida, fácil y económica de realizar en comparación con los procedimientos alternativos para el análisis de ADN. Además, consideramos que la técnica de RAPD es suficientemente robusta para ser aplicada como una técnica analítica de ADN para investigaciones epidemiológicas de rutina (Martinez *et al.*, 2000; Brandolini *et al.*, 2014; Nanayakkara *et al.*, 2018). Si bien, Martinez *et al.* (2000) sugieren que la tipificación RAPD generada por la combinación de cebadores OPS11, OPB17 y OPB18 aumenta la capacidad de la metodología para detectar la variabilidad entre aislamientos, a partir de los valores obtenidos para D y los patrones de bandas observados en el presente trabajo, sostenemos que la aplicación individual del cebador OPS11 es capaz de diferenciar los pares de cepas madre-recién nacido.

Como se expuso anteriormente, la técnica de PFGE continúa siendo la técnica *gold* estándar elegida para estudios epidemiológicos, por lo que también fue incluida en nuestro análisis (Åberg *et al.*, 2019). En este sentido, la obtención de resultados similares por medio de las técnicas RAPD y PFGE aplicadas en el presente trabajo, nos permite proponer a la técnica RAPD como una alternativa para su aplicación en laboratorios de baja y mediana complejidad.

Finalmente, cabe destacar la importancia y trascendencia desde un enfoque clínico-epidemiológico de estos aislamientos parentales debido a la escasez de situaciones diagnósticas que permiten recuperar SGB en simultáneo tanto de la madre como de su recién nacido. Esto se debe fundamentalmente a que a partir de la implementación de la búsqueda obligatoria de SGB por Ley Nacional N° 26.369 en toda mujer embarazada entre 35-37 semanas de gestación, si se detecta colonización materna se implementa la PIP y se previene la infección neonatal. Por lo tanto, los cuatro aislamientos parentales recuperados en este estudio son valiosos y representativos para evaluar la transmisión vertical.

Este es el primer estudio en la región y en el país que ha optimizado la técnica RAPD para su aplicación en el estudio de SGB dentro de centros de salud y universidades de Argentina y países vecinos como Paraguay y Brasil. A futuro se espera que esta técnica sea reproducida en otros laboratorios para la obtención de un mayor número de datos epidemiológicos que permitan estudios más complejos sobre dispersión clonal de cepas de SGB en embarazadas e infecciones invasivas en recién nacidos.

## CONCLUSIONES

Se seleccionó el cebador OPS11 por su robustez y capacidad para discriminar cepas de SGB no relacionadas a partir de la técnica RAPD.

Se confirmó la transmisión vertical y la relación clonal de SGB de las madres estudiadas a sus respectivos recién nacidos, por técnica de RAPD y por PFGE, que es la técnica de referencia, encontrando perfiles de bandas idénticos para cada par de aislamientos madre-recién nacido.

Se encontraron condiciones apropiadas para la técnica RAPD, que resulta de utilidad para realizar estudios epidemiológicos ligados a la portación materna de SGB y su transmisión al recién nacido, así como a la eventual transmisión horizontal de esta bacteria en centros de salud.

## ASPECTOS ÉTICOS-REGULATORIOS

Este trabajo fue aprobado por el Comité Científico del Hospital Central "Dr. Ramón Madariaga" de la ciudad de Posadas, Misiones y por el Comité Institucional de Ética de la Investigación en Salud de los Hospitales Materno Provincial, Materno Neonatal y Misericordia de la ciudad de Córdoba, Córdoba.

Se obtuvo consentimiento informado por escrito para cada paciente y datos médicos confidenciales según protocolo de estudio: C10 "Prevalencia de colonización vaginal y rectal de *Streptococcus* betahemolítico grupo B (SGB o *Streptococcus agalactiae*) en mujeres embarazadas de 35-37 semanas de gestación". Este estudio sólo revisó a pacientes humanos, ningún animal participó en ningún aspecto del estudio.

Los autores afirman que conocen las normas bioéticas vigentes en la Argentina y en la Declaración de Helsinki y sus enmiendas.

## AGRADECIMIENTOS

A los integrantes de la Cátedra de Bacteriología de la Facultad de Ciencias Exactas, Químicas y Naturales de la Universidad Nacional de Misiones, especialmente a su ex Profesora Titular la Mgter. Marta Vergara por ser pionera en este tema en la provincia de Misiones. A la Mgter. Viviana Villalba, Jefa del Servicio de Bacteriología del Laboratorio del Hospital Escuela de Agudos "Dr. Ramón Madariaga" por su colaboración en el aislamiento e identificación de las cepas de *Streptococcus agalactiae* recuperadas de neonatos.A la Dra. Adriana Limansky, Profesora Asociada de Bacteriología del Instituto de Biología Molecular y Celular de Rosario, Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, por su asesoramiento en la puesta a punto y ejecución de la técnica de RAPD.

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BAG | Journal of Basic & Applied Genetics

Vol XXXIII (2): 45-53; December 2022

Journal of the Argentine Society of Genetics

# SELECCIÓN CONTRA DISPLASIA DE CADERA CANINA EN EL OVEJERO ALEMÁN

# BREEDING AGAINST CANINE HIP DYSPLASIA IN THE GERMAN SHEPHERD DOG

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**Cite this article as:**
Poverene M.M. 2022. BREEDING AGAINST CANINE HIP DYSPLASIA IN THE GERMAN SHEPHERD DOG. BAG. Journal of Basic and Applied Genetics XXXIII (2): 45-53.

Received: 10/24/2022
Revised version received: 11/22/2022
Accepted: 11/23/2022
General Editor: Elsa Camadro
DOI: 10.35407/bag.2022.33.02.05
ISSN online version: 1852-6233

## ABSTRACT

Canine hip dysplasia (CHD) is a progressive and disabling disorder in large dog breeds, such as the German Shepherd dog. Breeding sires and dams free of dysplasia is the only way to reduce its incidence. Several diagnostic methods have been developed based on radiographic examination, on the basis of which dogs are selected for breeding. CHD has a polygenic hereditary basis and environmental influence, with a median to low heritability (*ca.* 0,20 to 0,40), so the progress in phenotypic selection has been slow. In Argentina, the prevalence of dysplasia in German Shepherd dogs remains high (> 25%) and it is impossible to predict its incidence in the offspring of the breeding stock. Some countries have implemented a selection based on the estimated breeding value, obtaining an important advance. Genome-wide association studies have revealed numerous CHD-associated markers and several candidate genes have been found that point to the possibility of implementing genomic selection in the near future.

Palabras clave: canine hip dysplasia, German Shepherd dog, phenotypic selection, genomic selection, estimated breeding value.

## RESUMEN

La displasia de cadera canina o displasia coxo-femoral (DCF) es un desorden progresivo e incapacitante en perros de razas grandes, como el Ovejero Alemán. La selección de reproductores libres de displasia es la única forma de reducir su incidencia. Se han desarrollado varios métodos de diagnóstico basados en el examen radiográfico, en base a los cuales se seleccionan los reproductores para la cría. La DCF tiene una base hereditaria poligénica e influencia ambiental, con una heredabilidad media a baja (alrededor de 0,20 a 0,40), por lo que el progreso de la selección fenotípica ha sido lento. En Argentina la prevalencia de la displasia en la raza sigue siendo alta (>25%) y es imposible prever su incidencia en la progenie del plantel de cría. Algunos países han implementado la selección basada en el valor estimado de cría, obteniendo un importante avance. Los estudios de asociación del genoma completo han revelado numerosos marcadores asociados a la DCF y se han encontrado varios genes candidatos que señalan la posibilidad de implementar una selección genómica en un futuro cercano.

Key words: displasia coxo-femoral, Ovejero Alemán, selección fenotípica, selección genómica, valor estimado de cría.

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La displasia de cadera es uno de los desórdenes musculo-esqueléticos más frecuentes en los perros y fue descrita por primera vez en 1935 (Schnelle, 1959). Displasia significa "crecimiento anormal" y es una alteración del desarrollo de la articulación coxo-femoral. A diferencia de la humana, la displasia de cadera canina no es congénita, sino que aparece durante el crecimiento (Ginja *et al.*, 2015). El fenotipo está caracterizado por la laxitud de la articulación de la cadera en perros jóvenes. Esa laxitud determina el movimiento lateral de la cabeza del fémur fuera del acetábulo a medida que aumenta el peso del perro, lo que produce un degaste anormal de las superficies óseas. Con la edad, tal condición a menudo lleva al desarrollo de osteoartrosis y osteoartritis secundaria en una o ambas caderas. La displasia coxo-femoral (DCF) causa inestabilidad, dolor y renguera clínica.

El Ovejero Alemán es especialmente susceptible a la DCF, con riesgos de invalidez y muerte por eutanasia que cuadriplican los de otras razas, probablemente debido a su conformación y postura como también por ser una raza de trabajo en la que la DCF no amerita el adiestramiento necesario (Zorko *et al.*, 2007; Malm *et al.* 2010). La prevalencia de este desorden en la raza a nivel mundial es de 35% (Lewis *et al.*, 2013). La DCF y la osteoartrosis son irreversibles y la única manera de aumentar el bienestar de las razas afectadas es la selección de reproductores que no presentan esta enfermedad.

### *Selección fenotípica*

El diagnóstico de DCF se basa en la observación de radiografías (Ginja *et al.*, 2010; Butler y Gambino, 2017). La técnica radiográfica está estandarizada en todo el mundo (Flückiger, 2007a). En el Ovejero Alemán la radiografía se realiza a partir de los 12 meses de edad. El grado de displasia se determina en base al grado de distorsión de la articulación coxo-femoral (Tabla 1). El criterio más importante es el ángulo de Norberg, que se define por una recta que une los centros de las cabezas de fémur derecha e izquierda y otra recta que une cada cabeza de fémur con el borde craneal del acetábulo. Ese ángulo en una cadera normal mide unos 105°.

El método de evaluación de DCF propuesto en 1984 por la *British Veterinary Association* (BVA) junto con el *Kennel Club* (KC, Reino Unido y Australia), y adoptado por otros países, se basa en nueve caracteres visibles en la radiografía, a los cuales se da un puntaje de 0 a 6 (donde 0 es normal y 6 es la condición más grave) a cada una de las caderas del perro. Esos caracteres son: 1. Ángulo de Norberg; 2. Subluxación; 3. Borde acetabular craneal; 4. Borde acetabular dorsal; 5. Contorno acetabular craneal efectivo; 6. Fosa acetabular; 7. Borde acetabular caudal; 8. Exostosis de la cabeza y cuello femoral; 9. Recontorneado de la cabeza del fémur. El puntaje máximo es de 53 por cada cadera, o sea que 106 puntos es la peor calificación que puede obtener un perro (Flückiger, 2007b).

Debido a la dificultad en cuantificar objetivamente los signos clínicos de la DCF, se han desarrollado diversas escalas radiológicas para que los criadores puedan seleccionar los perros más aptos para reproducción. Las más utilizadas son las de la Federación Cinológica Internacional (FCI, Europa), BVA y KC, y la *Orthopedic Foundation for Animals* (OFA, USA), pero, ya que consisten en evaluaciones subjetivas de las condiciones físicas del perro, no son fáciles de comparar (Tabla 2). El Club Argentino de Criadores del Perro Ovejero Alemán (POA) utiliza la misma escala que la *Verein für Deustche Schäfferhunde* (SV), la Asociación de Pastores Alemanes de Alemania.

Smith *et al.* (1990) introdujeron el concepto de laxitud de cadera pasiva versus funcional para distinguir entre subluxación evidente en la radiografía y la subluxación posterior inducida por la ganancia de peso durante el crecimiento. Desarrollaron un método radiográfico de estrés (radiografía de dis-tracción) que en 1993 originó el Índice de dis-tracción PennHIP (por el programa de mejoramiento de caderas de la Universidad de Pennsylvania, EUA). Este índice ha resultado ser un mejor predictor de la DCF y la osteoartrosis que el método de la FCI en varias razas, pero se ha utilizado limitadamente en la raza Ovejero Alemán (Leighton *et al.*, 2019).

La selección fenotípica en base al resultado radiográfico se implementó hace más de 60 años (Ginja *et al.*, 2015). La OFA estima que, en promedio, solo el 5% de los perros de pedigrí se somete a examen radiográfico (Reed *et al.* 2000). La radiografía es obligatoria en pocos países (Alemania, Australia y Suecia), en la mayoría es voluntaria y no hay requerimiento de control radiográfico en perros que no serán destinados a cría, por lo que los valores promedio de DCF publicados no reflejan el grado de prevalencia o severidad de la enfermedad dentro de una raza (Soo y Worth, 2015).

La DCF es considerada un carácter cuantitativo porque puede ser medida y clasificada en grados, desde normal a grave. Es un desorden multifactorial causado por herencia poligénica, factores ambientales y probablemente epigenéticos (King, 2017; Ohlerth *et al.*, 2019). La diferente expresión del desorden en las caderas derecha e izquierda es un efecto no genético y siempre se clasifica al perro por el fenotipo de la peor cadera.

Entre los factores ambientales relacionados con la DCF se encuentran la alimentación, la tasa de crecimiento y el ejercicio. La alta ingesta calórica, exceso de proteínas y de calcio, alta tasa de crecimiento, el sobrepeso, el exceso de ejercicio o ejercicios inadecuados son factores incidentes en perros con predisposición genética, pero sin ella los factores ambientales por sí mismos no pueden causar DCF. La castración temprana de hembras y machos también favorece la aparición de displasia,

**Tabla 1.** Clasificación del grado de displasia coxo-femoral (DCF) según la Federación Cinológica Internacional (Modificado de Flückiger, 2007a)

| Grado de DCF | Fenotipo |
|---|---|
| A | Sin signos de DCF<br>Cabeza del fémur congruente con el acetábulo. Borde cráneo-lateral del acetábulo redondo y bien definido. Espacio articular estrecho y uniforme. Ángulo de Norberg (AN) cercano a 105°. |
| B | Articulación de cadera casi normal<br>La cabeza del fémur y el acetábulo son ligeramente incongruentes y el AN es cercano a 105° o bien la cabeza del fémur es congruente con el acetábulo y el AN es menor de 105°. |
| C | DCF leve<br>La cabeza del fémur y el acetábulo son incongruentes, el AN es de unos 100° y/o hay un ligero achatamiento del borde acetabular cráneo-lateral. Puede haber escasos signos de osteoartrosis en los bordes acetabulares craneal, caudal o dorsal de la cabeza y cuello del fémur. |
| D | DCF moderada<br>Incongruencia obvia entre el acetábulo y la cabeza del fémur con subluxación. El AN es mayor de 90°. Achatamiento del borde cráneo-lateral y/o signos de osteoartritis presentes. |
| E | DCF severa<br>Marcados cambios displásicos en la articulación de la cadera, tales como subluxación evidente o luxación. El AN es menor de 90°. Obvio achatamiento del borde acetabular cráneo-lateral, deformación de la cabeza del fémur (forma de hongo o achatado) u otros signos de osteoartrosis. |

mientras que la administración de glucosamina-glucanos como polisulfatos, la previene (King, 2017).

Es notable que a partir del año 2000 en distintos países se observó un mejoramiento significativo del efecto combinado de factores no genéticos (alimentación, ejercicio) y este resultado favorable se debe a estrategias de manejo exitosas de criadores, veterinarios y dueños de los perros.

Reed *et al.* (2000) analizaron la influencia del padre, la madre e interacción padre x madre como factores aleatorios sobre la variación en los puntajes de conformación de la cadera de progenies de distintas razas. Tanto el macho como la hembra tuvieron un efecto significativo en la herencia de la DCF, pero la interacción macho x hembra no resultó significativa. Eso significa que la contribución genética del padre y de la madre es igual en la determinación de este desorden. Sin embargo, un macho puede transmitir sus características a un número mucho mayor de descendientes, especialmente aquellos machos populares entre los criadores.

La heredabilidad estimada de la DCF puede variar según la muestra de perros que se considere, el método de evaluación, la consanguinidad, los factores ambientales y el método de cálculo. En el Ovejero Alemán las estimaciones son de 0,22-0,43 según la OFA, 0,24-0,35 según la FCI y 0,30-0,35 según la BVA, aunque Leighton *et al.* (2019) han encontrado valores de heredabilidad mucho mayores (0,47 a 0,81). En un estudio sobre más de 13 mil Ovejeros Alemanes en Australia se encontró que la heredabilidad de los nueve caracteres analizados radiográficamente (método de BVA, 1984, antes descrito) iba de 0,14 a 0,24, siendo más alta en los tres primeros (0,23-0,24 para AN, subluxación y borde acetabular craneal) que en los restantes. Los primeros tres parámetros se corresponden con los rasgos de laxitud de la articulación y acetábulo poco profundo, mientras que los restantes se relacionan con los cambios osteoartrósicos (Wilson *et al.*, 2012).

La importancia práctica de dichas estimaciones de heredabilidad consiste en que, siendo el rasgo heredable,

**Tabla 2.** Comparación de distintos sistemas de clasificación de la displasia coxo-femoral, DCF (Modificado de Flückiger, 2007b)

| FCI[1] | Alemania excepto SV[2] | Alemania SV (Raza Ovejero Alemán) | OFA[3] (USA) | BVA/KC[4] (UK y Australia)[7] |
|---|---|---|---|---|
| A, normal | A1 | A, normal | Excelente | 0-4 (≤3 en cada cadera) |
| | A2 | A, normal | Bueno | 5-10 (≤6 en cada cadera) |
| B, límite | B1 | A, fast normal[5] | Bueno | 11-18 |
| | B2 | A, fast normal | Justa | 19-25 |
| C, leve | C1 | A, noch zugelassen[6] | Límite | 26-35 |
| | C2 | A, noch zugelassen | Leve | |
| D, moderada | D1 | D1 | Moderada | 36-50 |
| | D2 | D2 | Moderada | |
| E, severa | E1 | E1 | Severa | 51-106 |
| | E2 | E2 | Severa | |

[1]Federación Cinológica Internacional; [2]SV= Verein für Deustche Schäfferhunde, Asociación de Pastores Alemanes de Alemania; [3]Orthopedic Foundation for Animals; [4]British Veterinary Association y Kennel Club; [5]fast normal = casi normal; [6]noch zugelassen = todavía permitido; [7]el puntaje es la suma de ambas caderas.

sería posible disminuir su prevalencia a través de la selección. Cuanto mayor sea la heredabilidad, mayor será el avance genético que se obtendrá. La precisión de la selección fenotípica usando información del propio individuo equivale a la raíz cuadrada de la heredabilidad, de modo que, según los valores anteriores, la precisión de la selección estaría entre 0,47 (√0,22) y 0,65 (√0,43) si se consideran los valores estimados por OFA. Este es un rango de valores similar al de los caracteres de producción del ganado lechero, donde se ha obtenido un extraordinario avance genético en las últimas décadas (Wilson *et al.*, 2012).

Varios autores han señalado que a pesar de que el uso del examen radiográfico lleva más de 40 años, la prevalencia de la DCF permanece alta y han cuestionado los esquemas de selección utilizados por la FCI, OFA y BVA. Si bien se ha reducido el número de perros severamente afectados, ha habido sólo un progreso limitado en la reducción del impacto total de la DCF (Leighton, 1997; Wilson *et al.*, 2011; Oberbauer *et al.*, 2017; Ohlerth *et al.* 2019; Babá *et al.*, 2019; James *et al.*, 2020).

En la Figura 1 se muestran los resultados de placas de cadera en Argentina durante los últimos 14 años (datos tomados de POA), calificadas de acuerdo al sistema establecido por la SV alemana (Tabla 2). Las caderas Normal aumentaron un 10% y las Casi Normal un 8% en ese período. Las categorías Grave y Mediana disminuyeron un 4%, mientras que Todavía Permitido disminuyó un 11%. Eso implica que en ese período la displasia disminuyó de 43 a 24%, incluyendo a los perros de esa última categoría, a los que se permite competir y criar. Entre los criadores de todos los países existe una difundida costumbre de realizar una radiografía no oficial (llamada "preplaca") y de acuerdo al resultado, proceder o no a realizar la radiografía oficial. En consecuencia, es de esperar que la prevalencia de la DCF en Argentina sea aún mayor del 24%. Las categorías Mediana y Grave solo disminuyeron un 8%. Estos resultados podrían explicarse porque hasta el año 2011 los machos autorizados para criar eran de las categorías Normal, Casi Normal y Todavía Permitido, pero había todo tipo de hembras en el plantel de cría (http://clubpoa.org.ar/busca-tu-cachorro/) y aún en 2019 figuraba alguna hembra Mediana. Ya que la DCF tiene una probabilidad de heredarse de la misma forma tanto por parte del padre como de la madre, admitir hembras con displasia en el plantel de cría determinaría un diferencial de selección muy bajo. Desde diciembre 2019 solamente pueden criar los ejemplares con placa de displasia A (Normal, Casi Normal y Todavía Permitido) según el Reglamento de Crianza de POA (http://clubpoa.org.ar/reglamento-de-crianza-y-registro/). Aun así, de acuerdo con la Tabla 2, Casi Normal equivale al 25% del puntaje de BVA, y Todavía Permitido, al 33% de ese puntaje.

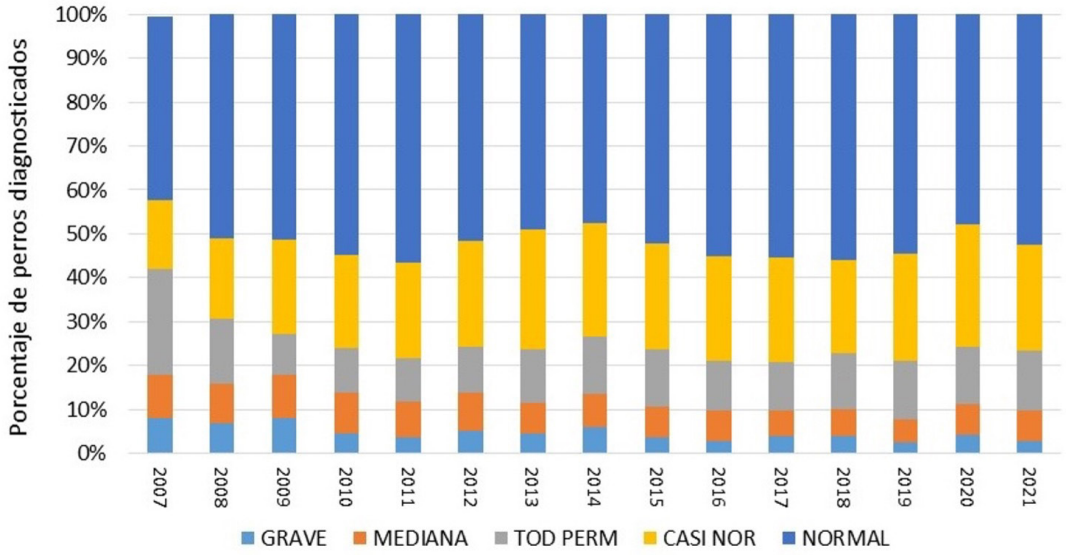


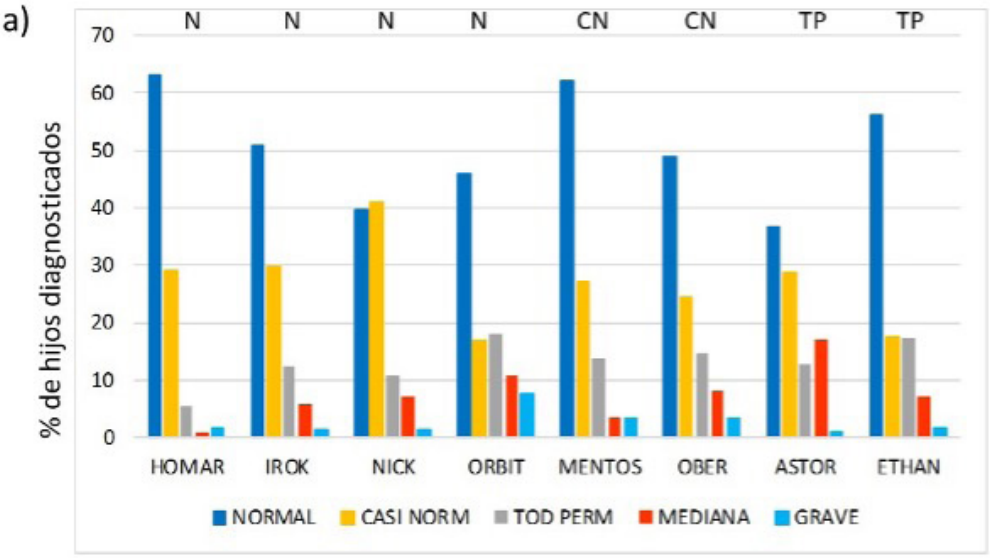

**Figura 1.** Frecuencia de las categorías de displasia coxo-femoral (DCF) en el período 2007 – 2021 en el Ovejero Alemán en Argentina. Datos tomados del Club Argentino de Criadores del Perro Ovejero Alemán, POA (http://clubpoa.org.ar/estadisticas/). Las categorías provienen del resultado de las placas radiográficas para el diagnóstico de DCF y son: Normal, Casi normal, Todavía permitido, Mediana, Grave.

### *Selección basada en el valor estimado de cría*

Varios autores sugieren que el progreso genético en DCF basado en los puntajes radiográficos sería mucho más efectivo si la selección basada en el valor de cría reemplazara a la selección fenotípica (Wilson *et al.*, 2012; Lewis *et al.*, 2013; Wilson y Nicholas, 2015; Leighton *et al.*, 2019; Wang *et al.*, 2019). El valor estimado de cría (en inglés, *Estimated Breeding Value, EBV*) indica el valor de un perro como reproductor, para determinado rasgo. El *EBV* se calcula a partir del fenotipo de un individuo, el de sus padres y sus relaciones de pedigrí. El fenotipo de su progenie resulta más informativo que el del individuo mismo, ya que perros con caderas normales pueden llevar alelos que determinen DCF en sus descendientes. La observación de la progenie de ocho campeones argentinos muestra que el fenotipo por sí solo no indica el riesgo de transmitir la DCF a su progenie (Figura 2a). La descendencia de los reproductores argentinos con más de 50 hijos diagnosticados, asumiendo para todos ellos que las hembras que sirvieron son una muestra al azar en cuanto a su fenotipo de DCF, muestra que es imposible predecir el resultado y tampoco relacionarlo con el fenotipo del perro. Así, sumando los hijos Normal y Casi Normal, un perro Casi Normal produjo una descendencia mejor en promedio que algunos Normal, mientras que un perro Normal tuvo un resultado más pobre que los Casi Normal y Todavía Permitido (Figura 2b). El análisis de los datos de POA señala la imperiosa necesidad de implementar otro tipo de selección en Argentina, ya que el riesgo de desarrollar DCF es muy alto para una raza que en 2021 registró unos 3.300 cachorros de pedigrí, pero ha llegado a registrar 10.000 una década atrás (http://clubpoa.org.ar/busca-tu-cachorro/listado-de-servicios-y-nacimientos).

La dificultad del avance genético con base solamente en el fenotipo del individuo reafirma que la selección sería mucho más efectiva basada en el valor de cría, o sea si la selección genotípica reemplazara a la selección fenotípica. En Australia comenzó a aplicarse este método en 1980 basado en el índice PennHIP de dis-tracción, antes descrito, teniendo en cuenta el puntaje individual del perro, el de todos los medios hermanos paternos y maternos y el de toda su progenie, si la había. Sobre más de 5.200 Ovejeros Alemanes, en la generación 7 más del 97% tenía caderas calificadas como Excelente (Leighton *et al.*, 2019). En Alemania, la SV comenzó a aplicar la selección basada en *EBV* en 1998, tomando en cuenta al individuo, sus padres y su progenie más un factor de ponderación que incluye factores genéticos, de género (hembra o macho), de ambiente y de heredabilidad que permiten corregir la información fenotípica. Para cada ejemplar se obtiene un número de Zuchtwert (HD ZW) o estimación del valor de cría para DCF, que es público y permite a los criadores elegir un reproductor (https://www.sv-doxs.net/). En esta raza se calcula que la selección basada

a)




b)

| Homar | Irok | Nick | Orbit | Mentos | Ober | Astor | Ethan |
|---|---|---|---|---|---|---|---|
| 92% | 80,5% | 80,6% | 63% | 89,2% | 73,5% | 65,4% | 73,6% |
| (1225) | (1468) | (306) | (220) | (336) | (226) | (393) | (350) |

**Figura 2.** a) Clasificación de la descendencia de reproductores con más de 50 hijos diagnosticados. Datos tomados del Club Argentino de Criadores del Perro Ovejero Alemán, POA (http://clubpoa.org.ar/estadisticas/). Sobre cada reproductor figura su propia calificación del grado de displasia coxo-femoral (DCF): N= Normal, CN= Casi Normal, TP= Todavía Permitido; b) Porcentaje de hijos Normal y Casi Normal correspondiente a cada reproductor. Entre paréntesis figura el número de hijos diagnosticados.

en *EBV* ha sido tres veces más efectiva que la selección fenotípica en términos de progreso genético (Stock *et al.*, 2011; Soo y Worth, 2015). Este hecho es importante porque Alemania tiene una población superior a 450.000 perros registrados y exporta reproductores a todos los países. Suecia y Finlandia también han implementado la selección basada en *EBV* en la raza Ovejero Alemán desde 2012 (Hedhammar, 2020).

### *Hacia una Selección genómica*

Desde 2004 se dispone de la secuencia de un genoma canino de referencia (Ostrander y Wayne, 2005; https://research.nhgri.nih.gov/dog_genome/) abriendo la posibilidad de identificar los genes responsables de la DCF (Guo *et al.*, 2011). Cada raza investigada ha mostrado diferentes mutaciones asociadas con la displasia (Zhou *et al.*, 2010; Oberbauer *et al.*, 2017). El uso de distintas escalas para el fenotipado y el escaso número de perros diagnosticados con respecto a la población general han dificultado el análisis de los genes involucrados (Stock *et al.* 2011).

Janutta *et al.* (2006) encontraron evidencia de un gen mayor, con escasa influencia ambiental, responsable del desarrollo de DCF en el Ovejero Alemán. Estos autores estudiaron 20 familias de Ovejeros Alemanes durante varias generaciones, incluyendo más de 8.500 perros, encontrando que el modelo que mejor ajustó a los datos fue una combinación de genes mayores con poligenes. Esto explicaría la dificultad en erradicar esta dolencia. Los primeros estudios de asociación del genoma completo (GWAS) en la raza se realizaron en Alemania, ya que la SV dispone de estudios radiográficos, muestras de ADN y registros completos de pedigrí de un gran número de ejemplares. Marshall y Distl (2007) hallaron QTL asociados con la DCF en nueve de los 39 pares de cromosomas (Tabla 3). Posteriormente, 19 QTL pudieron relacionarse con distintos genes candidatos (Fels y Distl, 2014; Fels *et al.* 2014). Diez de esos genes están relacionados entre sí y constituyen una red asociada a la formación de hueso y cartílago. Otro gen

**Tabla 3.** QTL y genes candidatos asociados con fenotipos de displasia coxo-femoral descritos por el método de FCI en el Ovejero Alemán.

| Origen y tamaño de la población analizada | Cromosomas (CFA) donde mapean los QTL | Genes candidatos posicionales con SNP dentro de los QTL hallados | Referencia |
|---|---|---|---|
| Alemania (459) | 1, 3, 4, 8, 9, 16, 19, 26, 33 | n/a | Marshall y Distl, 2007 |
| Alemania (834) | 3, 9, 26, 33, 34 | *PGM2, PCNP, TRIO, EPHA3-6, SEMA5A, SLCGA3, FGF12* | Fels *et al.*, 2014 |
| Alemania (1035) | 19, 24, 26, 33, 34 | *SRC, KSR2, TRIO* | Fels y Distl, 2014 |
| Finlandia (750) | 1, 9, 25, 28 | *NOX3, ARID1B, NOG, NANOS1* | Mikkola *et al.*, 2019a |
| Finlandia (531) | 1, 9 | *NOX3, ARID1B, RNF43* | Mikkola *et al.*, 2019b |
| Reino Unido (180) y Suecia (402) | 9, 21 | *MED13, PLEKHA7* | Wang *et al.*, 2021 |


**Figura 3.** Esquema de los pasos a seguir para obtener el valor de cría (GEVB) necesario para realizar una selección genómica en contra de la displasia coxo-femoral (DCF) en la raza Ovejero Alemán. La ecuación predictiva daría valores a cada uno de los fenotipos de DCF, desde Normal a Grave.

en el cromosoma 24 asociado a las diferencias entre perros libres de DCF y afectados con grados C a E (Tabla 2), está relacionado con la formación de hueso (Fels y Distl, 2014). Mikkola *et al.* (2019a) encontraron dos SNP en el cromosoma canino (CFA) 1 y otros dos en el CFA 9 cuyos alelos se corresponden con fenotipos libres o leves de DCF, o bien con fenotipos de DCF moderada a severa. En el CFA 9 uno de ellos coincide con el gen *NOG*, que codifica una proteína necesaria para el desarrollo del tubo neural y para la condrogénesis, osteogénesis y formación de articulaciones en el embrión. *NOX3* en el CFA 1 es un gen candidato para la degradación del cartílago articular. Las variantes en el CFA 1 se asociaron a la presencia o ausencia de signos radiográficos de osteoartrosis, mientras que loci en los CFA 9 y 28 se correspondieron con signos de incongruencia de la articulación de la cadera (Mikkola *et al.*, 2019b). Sin embargo, asociaciones entre distintos fenotipos de displasia y numerosos SNP en poblaciones de Ovejeros Alemanes del Reino Unido, Suecia y Finlandia confirman la naturaleza genética compleja de la DCF, con múltiples loci asociados al rasgo (Wang *et al.*, 2021). Cuatro SNP con

asociación significativa con la DCF resultaron comunes a varias razas estudiadas (entre ellos, dos en los CFA 1 y 26 descritos en el Ovejero Alemán, Tabla 3) pero muchas más asociaciones significativas con SNP se encontraron dentro de cada raza (Mikkola *et al.*, 2021) confirmando la arquitectura genética compleja de la DCF y del genotipo de todos los genes relacionados con ella en las diversas razas.

La selección genómica se basa en un análisis de ADN que provea ciertos marcadores en desequilibrio de ligamiento con genes asociados con la DCF. Ya que el análisis puede realizarse al nacimiento, la selección genómica representaría un avance comparada con la selección basada en *EBV* (Sánchez Molano *et al.*, 2014). En la raza Labrador se ha desarrollado un modelo basado en siete polimorfismos genéticos con alto valor predictivo para la detección temprana de la DCF; seis de estos SNP se han localizado en genes candidatos relacionados con el metabolismo de la matriz extracelular y con el metabolismo del hueso (Bartolomé *et al.* 2015) y coinciden con los genes candidatos hallados en el Ovejero Alemán por Fels y Distl (2014). También se ha investigado el tamaño de la población de referencia que permitiría aplicar la selección genómica en la raza Labrador (Edwards *et al.*, 2018).

El modelo predictivo de DCF desarrollado por Bartolomé *et al.* (2015) para la raza Labrador no ha sido probado en otras razas. Los QTL que influencian la DCF en el Ovejero Alemán han mostrado convergencia en su ubicación en diversos estudios. Disponer de marcadores en desequilibrio de ligamiento con esos genes permitiría caracterizar cada uno de los grados de DCF en una población de referencia y desarrollar una ecuación predictiva, que se aplicaría luego al diagnóstico de reproductores y camadas (Figura 3). La ecuación predictiva permitiría realizar un diagnóstico de DCF con base en los marcadores de ADN en una edad temprana, sin necesidad de esperar 12 meses para evaluar radiográficamente al ejemplar, y determinar su valor de cría genómico (*GEBV*). El reto que queda es la identificación de las mutaciones que colectivamente sustentan la DCF y la osteoartrosis para entender la patogenia molecular de estas enfermedades. Cuando los genes que más significativamente influyen en esta dolencia sean localizados mediante marcadores moleculares, la selección genómica será una alternativa valiosa para la raza Ovejero Alemán.

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Vol XXXIII (2): 55; December 2022
Journal of the Argentine Society of Genetics

OBITUARIO - OBITUARY

# DR. ENRIQUE CURT GADOW

30/07/1938 - 07/11/2022

Nació en Apóstoles, Misiones, Viudo de María del Carmen Pérez, deja dos hijas: Fabiana y Andrea, y cinco nietos.

Médico por la Facultad de Medicina de la Universidad de Buenos Aires en 1962 y, al año siguiente, inició la formación de posgrado en el entonces Hospital de Nueva York, el actual Centro Médico Weill Cornell, hasta 1969. De regreso al país, se incorporó en 1970 al Centro de Educación Médica e Investigaciones Clínicas (Cemic), fue director de Investigación del Instituto Universitario y presidente del Comité de Ética en la Investigación.

Para los integrantes del CEMIC su pérdida significa mucho por su liderazgo, compromiso, pertenencia y profesionalismo. Fue un gran maestro y consejero, especialmente a quienes se desempeñan en el Departamento de Obstetricia y Ginecología y la Sección Genética Médica. Será recordado mundialmente y en nuestro país como pionero de la medicina materno fetal y genética.

En 1985, se doctoró en Medicina en la UBA. Desde 1998 ocupó como miembro de número el primer sitial en la historia de la Academia Nacional de Medicina consagrado a la especialidad Genética.

A lo largo de su carrera, Gadow presidió la Sociedad Argentina de Genética (1978-1980), la Sociedad Argentina de Ginecología y Obstetricia (1994-1995) y la Sociedad de Obstetricia y Ginecología de Buenos Aires (1995). En 1993, recibió el Diploma al Mérito en la categoría de Genética y Citología del Premio Konex a las mejores figuras de la ciencia y la tecnología de la década anterior. Su nombre figura entre los galardonados en 2002 con el Premio Maestro de la Medicina Argentina, auspiciado por *La Prensa Médica Argentina.*

**Angela R. Solano**

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