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Short running title:

"rs10974944 in Mexican populations and myeloproliferative diseases"

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Abstract

The genetic variant *rs10974944* (C>G) in the *JAK2* gene is associated with a higher risk of myeloproliferative neoplasms (MPNs) by increasing the probability of the somatic mutation V617F in the JAK2 protein. For this reason, we evaluated the distribution of *rs10974944* in Mexican populations, including published data from association studies in worldwide populations. We analyzed five Mestizo (admixed) (n= 200) and four Native American population samples from Mexico (n= 200), representing the North, Center, West, and South regions of this country. Therefore, we genotyped *rs10974944* by qPCR using Taqman probes. Allele and genotype frequencies were estimated in each population sample. The wild-type allele C, the homozygous C/C, and the heterozygous C/G were the most frequent in all Mexican populations. The genotype distribution in all these population samples were in Hardy-Weinberg equilibrium. Interestingly, genetic distances clustered most of the worldwide patient samples, including to Tarahumaras and Mayas, and they showed differences with Mexican and control samples. Although higher genetic susceptibility to MPNs could be predicted in these Native American populations, the homogeneous allele distribution among Mexican and worldwide control populations, compels to analyze further genetic and non-genetic factors. In brief, although worldwide population samples displayed homogeneous distribution for *rs10974944*, the genetic clustering of worldwide patients supports the claimed association with myeloproliferative neoplasms.

Keywords: SNPs; Myeloproliferative neoplasms; Mexican; Native American; JAK2

INTRODUCTION

The Janus kinase 2 (JAK2) gene is located on 9p24.1 (Hermouet & Vilaine, 2011; Meier et al. 2009) and encodes signaling homodimeric receptors, such as the erythropoietin receptor (EPOR), thrombopoietin receptor (MPL), granulocyte colony-stimulating factor (G-CSFR), and thrombopoietin (TPO). The JAK2 protein is also used by some heterodimeric receptors to increase cell proliferation and resistance to apoptosis (Trifa et al. 2010; Anelli et al. 2018; Torres et al. 2022).

The *JAK2* gene has different single nucleotide polymorphisms (SNPs) associated with myeloproliferative neoplasms (MPNs), including hematological cancers characterized by hyperplasia of one or more elements of the myeloid series (leukocytes, platelets, and red blood cells) with effective maturation, proliferation, and possible progression to medullary fibrosis or leukemic transformation (Trifa et al. 2010; Paes et al. 2022; Torres et al. 2022). The main disorders related to the *JAK2* gene are polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic or primary myelofibrosis (PMF) (Alabdulaali, 2009; Baumeister, Chatain, Sofias, Lammers, & Koschmieder, 2021; Baxter et al., 2005; Kilpivaara et al., 2009; Nielsen, Birgens, Nordestgaard, & Bojesen, 2013; Torres et al., 2022).

The somatic mutation V617F substitutes thymine (T) for guanine (G) at nucleotide 1849 (1849G>T) of exon 14 of the *JAK2* gene, which substitutes phenylalanine (F) for valine (V) at amino acid 617 of the JAK2 polypeptide (Trifa et al. 2018). This gain-of-function variant affects the auto-inhibitory activity of the JH2 domain that results in constitutive activation of the JAK-STAT pathway and interferes with intracellular signaling (Hubbard, 2018). The V617F mutation causes the transformation of hematopoietic cells into cytokine-independent growth, promoting

tumorigenesis, tumor progression, and inflammation caused by continuous stimulation within the hematopoietic cells (Paes et al., 2022; Torres et al., 2022).

The germline risk variants rs3780367G (intron 10), rs10974944G (intron 12), rs12343867C (intron 14), and rs1159782C (intron 15) form the “GGCC” haplotype and are described as haplotype “46/1” (Anelli et al., 2018; Paes et al., 2022; Torres et al., 2022). This haplotype is one of the possible “pre-JAK2V617F” events that reportedly increases the risk of MPNs three to four times, and half of the risk is attributable to inherited factors (Hinds et al., 2016; Macedo et al., 2015; Paes et al., 2022). Among these SNPs, *rs10974944* was the first to be associated with the emergence of MPNs. Association studies conducted in Europe, Japan, China, North America, and Brazil have shown that rs10974944-G is more frequent in MPN patients -especially those that are V617F positive- than in the control population (Paes et al., 2022). The importance of r*s10974944* as a predictive marker for the V617F somatic event came from the study of 49,488 Danish individuals from the general population (Nielsen et al., 2013). They found that the fraction of individuals positive for the *JAK2* V617F somatic mutation increased across the r*s10974944* genotype as follows: 53% for homozygous C/C, 40% for heterozygous C/G, and 7 for homozygous G/G (*p*-trend= 0.001). More recently, the G/G genotype of *rs10974944* also has been associated as a genetic risk factor for MPN in the JAK2 V617F positive group in the Vietnamese population (Ngoc, Hau, Vuong, & Xuan, 2022). However, among Hispanic populations, such as Mexico, the distribution of the variant *rs10974944* is completely unknown.

The main Spanish-speaking population in Mexico is known as Mestizo (admixed) and constitutes ~93% of the total population (Rubi-Castellanos et al.

2009). The remaining Mexican population includes a more heterogeneous group involving Native Americans who live in rural and isolated geographic regions of the country (Martínez-Cortés, et al, 2013). These indigenous groups show larger genetic differentiation due to Pre-Hispanic genetic drift effects promoting increased frequencies for some genetic variants (Rangel-Villalobos et al. 2016; Ellegren & Galtier 2016). Interestingly, the admixture pattern in present-day Mestizos recapitulates the Native American substructure and can affect biomedical traits (Moreno-Estrada et al., 2014; Salazar-Flores et al., 2015). This may be the case for the risk-allele *rs10974944*-G that has shown a higher frequency in the American continent regarding worldwide populations (34 *vs*. 25%), according to the 1000 genomes project (http://www.ensembl.org/). Unfortunately, the genetic diversity of the variant *rs10974944* has been scarcely studied in Mestizos and Native Americans from Mexico to evaluate its possible biomedical impact.

In this paper, we describe the variant *rs10974944* of the *JAK2* gene in population samples of Mestizos and Native Americans from different regions of Mexico. We aimed to obtain a possible genetic risk landscape for MPNs based on *rs10974944* in this country and to integrate this knowledge into the previous worldwide reports.

MATERIALS AND METHODS

Population samples

For each Mexican population, we analyzed 50 DNA samples of indigenous volunteers from the following groups and geographic regions: i) Tarahumaras (North-Center), ii) Purepechas (West), iii) Nahuas (Center), and iv) Mayas (Southeast). In addition, we studied 40 DNA samples of Mestizo individuals from the following

Mexican states and regions: 1) Chihuahua (North-Center), 2) Nuevo Leon (North-East), 3) Jalisco (West), 4) Guerrero (South), and 5) Yucatan (Southeast) (Figure 1). DNA samples were taken from the genomic library of the Molecular Genetics Research Institute of the University of Guadalajara (UdeG), México. All participants were well-informed about the research purpose of their participation and signed a written informed consent under approval of the Committee of Ethics and Research of the Centro Universitario de la Ciénega (CUCI-UdeG), México. This study followed the regulations of the General Health Law on research involving human subjects (Mexico) and the Helsinki Declaration.

FIGURE 1

Genotyping

Total peripheral blood samples of the participants were collected for gDNA extraction using the salt-out DNA precipitation technique. DNA was quantified with the spectrophotometer NanoDrop 2000 (Thermofisher Scientific), and diluted with DNase-free water to a concentration of 20 ng/μL. Allelic discrimination assays were run to analyze the *rs10974944* variant on a QuantStudio 5 Real-Time PCR System using the following TaqMan probes under conditions provided by the supplier (Applied Biosystems, Thermo Fisher, USA): [VIC-“C”/FAM-“G”] CAGTCAGGTGGTGAGGGTTGATGAT[C/G]AGCCACATTTATCAAGGGGGTTAA G. The volumes *per* sample for the 10 μL qPCR were the following: 3 uL Master mix, 0.25 uL TaqMan probe, 4.75 uL H_2O, plus 2 μL of DNA sample. The thermocycler conditions included: i) initial denaturation at 95°C for 5 m; ii) 48 cycles at 92°C for 15 s and then 60°C for 60 s; and iii) final extension at 60°C for 3 m.

Statistical analysis

Descriptive statistics were calculated using the Microsoft Excel complement GenAlEx (Peakall & Smouse, 2012). We estimated allele and genotype frequencies, and expected heterozygosity (He) as genetic diversity parameters. We evaluated the Hardy-Weinberg equilibrium (HWE) by means of Fisher's exact tests with 5000 simulations using the Genetic Data Analysis software (GDA 1.1) (Lewis & Zaykin 2001). For the interpopulation analysis, we included previously reported genetic patient-control databases for *rs10974944* from some worldwide populations. We used the letter P to distinguish control samples (e.g. Spain) from patient samples (e.g. SpainP) (Table 1). We ran pairwise comparisons to obtain Fst *p*-values between populations, and Fst genetic distances with the Arlequin 3.5 software (Excoffier & Lischer, 2010), which were graphically represented in a Neighbor-Joining (NJ) dendrogram with Treeview (Page, 1996). We applied the Bonferroni correction as properly described in the results section. Finally, Analysis Molecular of Variance (AMOVA) was performed to evaluate genetic structure among populations by means of inbreeding coefficient computations, such as Fst values and their significance evaluation (*p*-values) in Arlequin software.

TABLE 1

RESULTS

Descriptive statistics

Allele and genotype frequencies for rs10974944 in the *JAK2* gene were estimated in five Mexican Mestizo and four Native American population samples from different geographic regions (Table 2). Although the wildtype allele C was most frequent in all the Mexican populations (57 to 76%), the genotype distribution was

not identical in all populations. The most frequent genotype for the northern (Chihuahua and Tarahumaras), and southeastern (Yucatán and Mayas) populations was the C/G heterozygote (Table 2). Conversely, the wildtype homozygous C/C homozygote was most frequent in all the remaining Mexican populations, while the risk-genotype G/G was -consistently- the least frequent in both Mestizo (2.5 to 15%) and Native American populations (6 to 20%) (Table 2). The genotype distribution of *rs10974944* was in agreement with the HWE expectations in all nine Mexican population samples ($p \geq 0.20$) (Table 2).

TABLE 2

Interpopulation analysis

For comparative purposes, we represented graphically the allele and genotype frequencies of *rs10974944* in the *JAK2* gene of Mexican and previous patient-control studies from worldwide populations (Figure 2). As observed in Mexican populations, the wild-type allele C was most frequent in most of the worldwide population samples, except in patients from China (ChinaP= 47.7%) and Brazil (BrazilP= 50%). However, the frequency of the risk-allele G and risk-genotype G/G were higher in patients than in the corresponding control population samples (Figure 2). In fact, the genotype G/G was at least two times more frequent in worldwide patients than in their corresponding population controls, except by Slovenia where patient and control groups reported the same frequency (8.9%) (Zerjavic, Zagradisnik, Lokar, & et al, 2013). These findings support the global association of this variant of *JAK2* gene with myeloproliferative neoplasms (MPNs). By continent, the range of frequency for the risk genotype G/G in European patients

(8.9 to 16.6%) was lesser than that observed in most Asian and Brazilian patients (22.7 to 32.1%), except in Taiwan (16.4%) (Figure 2).

FIGURE 2

We evaluated the genetic relationships between all Mexican and worldwide populations by means of Fst genetic distances and pairwise Fst *p*-values based on *rs10974944* in the *JAK2* gene (Supplementary Table S1). Although one moderate difference was observed between Tarahumaras and Mestizos from Nuevo Leon (MxNL) ($p< 0.01$), no significant difference was observed after Bonferroni correction among Mexican populations ($p< 0.0031$). This finding supports an interpopulation homogeneity for *rs10974944* in this country, which was confirmed by the non-significant differentiation of populations based on an AMOVA (Fst= 0.29; p= 0.27) (Table 3).

TABLE 3

The Mexican populations Tarahumara and Mestizos from Nuevo Leon (MxNL) were the most differentiated from seven worldwide populations ($p< 0.01$) (Supplementary Table S1); they were followed by Nahuas and Mestizos from Guerrero (MxGue) with five and four interpopulation differences, respectively. Interestingly, at a global level, the population samples that showed greater number of interpopulation differences ($p< 0.01$) involved patients from China (19), Vietnam (16), Japan (14), Romania (12), and Brazil (12) (Supplementary Table S1).

We estimated a significant worldwide interpopulation differentiation for *rs10974944* (Fst= 3.17%; p= 0.0000) (Table 3). However, most of this differentiation comes from patients (Fst= 1.93%; p= 0.000), but not from their corresponding population controls that was not significant (Fst= -0.03; p= 0.06). Although Mexican

Native Americans showed larger interpopulation differentiation than Mexican Mestizos (Fst= 0.88 vs -0.8, respectively), both were not significant ($p > 0.05$), which support the previous conclusion of genetic homogeneity for *rs10974944* in this country.

The genetic relationships based on *rs10974944* in the *JAK2* gene were represented in a NJ dendogram (Figure 3). As expected from the estimated pairwise Fst *p*-values (Supplementary Table S1), the largest branches of the tree include patient population samples in the upper right-side of the NJ tree, except those from Slovenia (SlovP). This finding is in agreement with the largest interpopulation differentiation of worldwide patients due to the high frequency of the risk-allele G. Interestingly, this "worldwide-patient" branch includes three of the four Mexican Native American groups analyzed herein: Tarahumaras, Mayas, and Purepechas (Figure 3). Conversely, most of the Mestizo populations were clustered with short branches in the middle and low branches of the tree.

FIGURE 3

DISCUSSION

The variant *rs10974944* was the first SNP of the *JAK2* gene to be associated with the emergence of myeloproliferative neoplasms (MPNs) in different worldwide populations (Table 1). Particularly, the genotype G/G has been associated with the somatic mutation V617F in MPN patients (Ngoc et al., 2022; Nielsen et al., 2013; Paes et al., 2022). However, population studies of *rs10974944* are scarce or absent in Latin America and Mexico, respectively.

We report here, for the first time, the allele and genotype frequency of the variant *rs10974944* in the *JAK2* gene in Mexican populations from different geographic regions (Table 1). In addition, we included interpopulation analyses with worldwide population datasets from control-patient studies available in the literature (Table 2). Although the 1000 Genomes Project describes frequencies for *rs10974944* in some worldwide populations (http://www.ensembl.org/), for comparison purposes we took into account association studies to get a better landscape of the possible biomedical impact in Mexican populations.

Our results showed a wide prevalence of the wildtype allele C in Mexican and worldwide populations, in addition to the prevalence of C/G heterozygotes and wildtype C/C homozygotes in our sample populations from Mexico (Figure 2). However, although worldwide patients showed a significant interpopulation differentiation (Fst 1.93; p= 0.000), their corresponding controls suggest a relatively homogeneous distribution when patients were omitted from this genetic structure population test.

The majority of patients from the worldwide population samples showed higher frequencies than their corresponding control samples for the risk allele G (average: 43.2 *vs* 28.5) and risk genotype G/G (average 9 *vs* 21.3) (Figure 2). In addition, patients from worldwide population samples showed a peculiar distribution in our NJ dendrogram regarding control and most of the Mexican population samples (Figure 3). Altogether, these findings also supported the global association of the variant *rs10974944* of the *JAK2* gene with myeloproliferative neoplasms (MPNs) (Pagliarini et al. 2013; Koh et al. 2014; Zerjavic et al. 2013; Soler et al. 2015; Hsiao et al. 2011; Matsuguma et al 2019; Trifa et al. 2016; Ngoc et al. 2022).

Interestingly, some Mexican Native American populations also showed elevated frequencies for the risk allele G (Tarahumaras 43% and Mayas 39%), and/or for the genotype G/G (Tarahumaras 20%, Purepechas 16%, and Guerrero 15%), similar to some worldwide patient population samples (Ngoc et al., 2022) (Figure 2). Although a higher risk of MPN could be attributed to some Mexican populations based on their higher frequencies of the G allele and G/G homozygote, caution is advised for the following reasons; 1) the presence of additional genetic factors is unknown in these Mexican populations, such as the haplotype that presumably increases the risk of MPNs three to four times, known as "pre-JAK2V617F", which is based on rs3780367-G, rs10974944-G, rs12343867-C, and rs1159782-C (Anelli et al., 2018; Paes et al., 2022; Torres et al., 2022), 2) the presence (or absence) of some non-genetic predisposing factors associated to MPNs in these Mexican populations is mostly unknown, mainly for the Native American populations, and 3) the consistent genetic homogeneity concluded for *rs10974944* of the *JAK2* gene among Mexican populations, as supported by the pairwise comparisons, genetic distances, and AMOVA (Table 3 and Supplementary Table S1).

CONCLUSIONS

The variant *rs10974944* of the *JAK2* gene showed a similar distribution among Mexican Native American and Mestizo populations compared to worldwide (control) population samples with some exceptions among the native American populations. Thus, the genetic risk of developing MPNs based on this SNP is predicted to be similar among the studied Mexican populations. Conversely,

worldwide population patients exhibited higher frequencies of the G risk allele than their corresponding control samples, which is in agreement with the previously claimed association of *rs10974944* with myeloproliferative neoplasms (MPNs).

Declarations

Authors declare no conflicts of interest with respect to the research, authorship, and publication of this article.

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Table 1. Description of the Mexican and worldwide populations used for interpopulation analysis for the SNP *rs10974944* of the *JAK2* gene.

Population	Abbreviation*	Sample size			
Mexican Native Americans		**n**		**Region**	**Reference**
Tarahumaras	Tarah	50		North-Center	This study
Purepechas	Purep	50		West	This study
Nahuas	Nahua	50		Center	This study
Mayas	Maya	50		Southeast	This study
Mexican Mestizos (admixed)		**n**		**Region**	**Reference**
Chihuahua	MxCh	40		North-Center	*This study*
Nuevo León	MxNL	40		North-East	*This study*
Jalisco	MxJal	40		West	*This study*
Guerrero	MxGue	40		West	*This study*
Yucatan	MxYuc	40		South-East	*This study*
Worldwide populations		**Control n**	**Patients n**	**Origin**	**Reference**
Brazil	Braz-BrazP	90	56	Maringa	(Pagliarini et al., 2013)
Spain	Spain-SpainP	270	129	Murcia	(Soler et al., 2015)
Romania	Rom-RomP	433	529	Bucharest	(Trifa et al., 2016)
Slovenia	Slov-SlovP	459	135	Maribor	(Zerjavic et al., 2013)
China	China-ChinaP	470	128	Hong-Kong	(Koh et al., 2014)
Japan	Jap-JapP	366	201	Yamaguchi	(Matsuguma & et al, 2019)
Taiwan*	Taiw-TaiwP	106	61	Kaohsiung	(Hsiao et al., 2011)
Vietnam	Viet-VietP	192	262	Hanoi	(Ngoc & et al, 2022)

*For abbreviations in worldwide patient-control studies, we used the letter P to distinguish patients from controls samples.

Table 2. Genotype and allele frequencies distribution of the *rs10974944* in the *JAK2* gene in Mexican Mestizos and indigenous groups.

Population	Genotype frequency n (%)			Allele frequency n (%)		HWE* *p*-value	He ¥
	CC	**CG**	**GG**	**C**	**G**		
Mestizo (admixed)							
Chihuahua	15 (37.5)	24 (60)	1 (2.5)	54 (67)	26 (33)	0.020	0.439
Nuevo León	20 (50)	19 (47.5)	1 (2.5)	59 (74)	21 (26)	0.151	0.387
Jalisco	20 (50)	15 (37.5)	5 (12.5)	55 (68)	25 (32)	0.421	0.43
Guerrero	22 (55)	12 (30)	6 (15)	56 (70)	24 (30)	0.071	0.42
Yucatán	17 (42.5)	20 (50)	3 (7.5)	54 (67)	26 (33)	0.377	0.439
Indigenous							
Tarahumaras	17 (34)	23 (46)	10 (20)	57 (57)	43 (43)	0.663	0.49
Purepechas	25 (50)	17 (34)	8 (16)	67 (67)	33 (33)	0.102	0.442
Nahuas	25 (50)	22 (44)	3 (6)	72 (72)	28 (28)	0.519	0.403
Mayas	17 (34)	27 (54)	6 (12)	61 (61)	39 (39)	0.340	0.476

*HWE: Hardy-Weinberg equilibrium. Significance level after Bonferroni correction: *p<0.0055*
¥ He= Heterozygosity expected (2pq)

Table 3. Analysis Molecular of Variance (AMOVA) to evaluate population structure among Mexican and worldwide populations for rs10974944 in the *JAK2* gene.

Population group	N *	Fst (%)	*p*-values	Within Pop
Worldwide	25	3.1686	0.000	96.83
Patients	8	1.9349	0.000	98.06
Controls	8	-0.0331	0.0606	100.03
Mexican Populations (Mx)	9	0.2901	0.2708	99.71
Native Americans (Mx)	4	0.8975	0.1222	99.10
Mestizos-admixed (Mx)	5	-0.8013	0.8153	100.8

* N: number of populations of the corresponding

FIGURE 1. Geographic location of the five Mestizo (admixed) and four Native American populations from Mexico analyzed in this work.

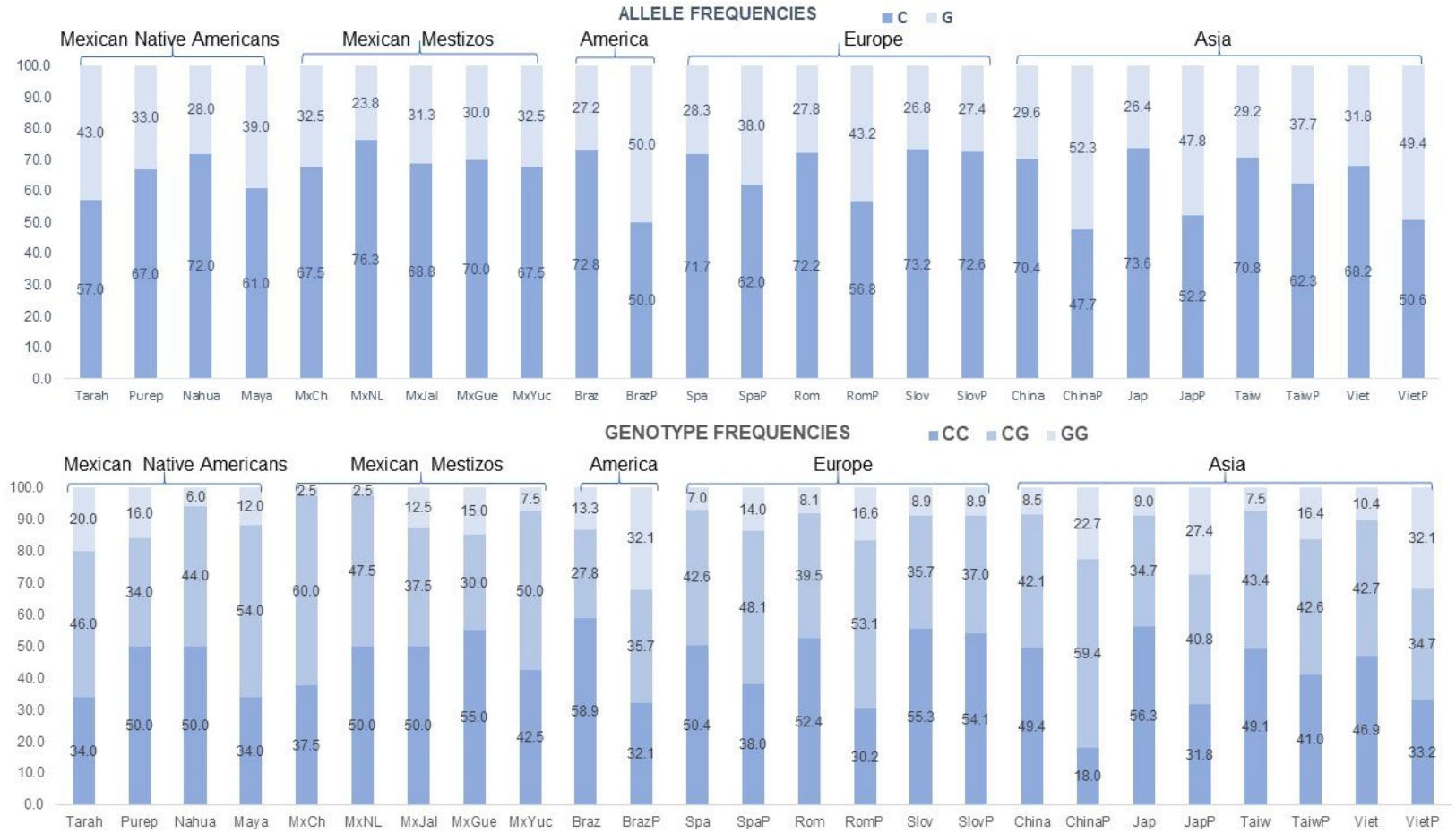

FIGURE 2. Allele and genotype distribution of the Mexican populations and published databases from worldwide association studies focused on *rs10974944* of the *JAK2* gene and myeloproliferative diseases. For abbreviations, please see Table 1**.** In population samples of patients, the letter P was added (e.g. Spain vs, SpainP).

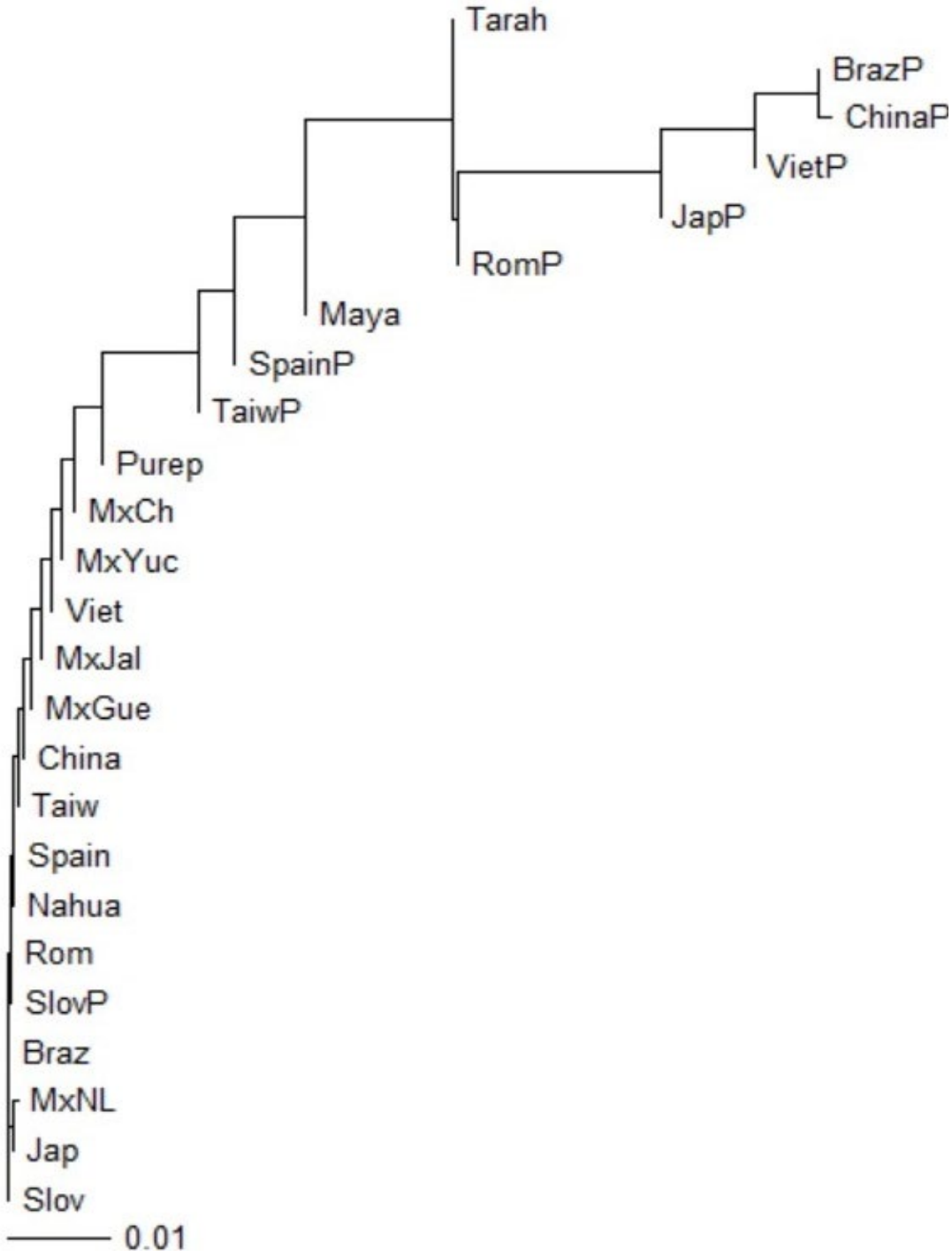

FIGURE 3. Neighbor Joining (NJ) dendrogram from Nei genetic distances based on *rs10974944* of the *JAK2* gene between 25 worldwide populations including those from Mexico analyzed herein. For abbreviations, please see Table 1. In population samples of patients, the letter P was added (e.g. Spain vs, SpainP).