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RESEARCH ARTICLE

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A tale of agriculturalists and hunter-gatherers: Exploring the thrifty genotype hypothesis in native South Americans

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Abstract

Objectives: To determine genetic differences between agriculturalist and hunter-gatherer southern Native American populations for selected metabolism-related markers and to test whether Neel's thrifty genotype hypothesis (TGH) could explain the genetic patterns observed in these populations.

Materials and Methods: 375 Native South American individuals from 17 populations were genotyped using six markers (*APOE* rs429358 and rs7412; *APOA2* rs5082; *CD36* rs3211883; *TCF7L2* rs11196205; and *IGF2BP2* rs11705701). Additionally, *APOE* genotypes from 39 individuals were obtained from the literature. AMOVA, main effects, and gene-gene interaction tests were performed.

Results: We observed differences in allele distribution patterns between agriculturalists and hunter-gatherers for some markers. For instance, between-groups component of genetic variance (F_{CT}) for *APOE* rs429358 showed strong differences in allelic distributions between hunter-gatherers and agriculturalists ($p = 0.00196$). Gene-gene interaction analysis indicated that the *APOE* E4/*CD36* TT and *APOE* E4/*IGF2BP2* A carrier combinations occur at a higher frequency in hunter-gatherers, but this combination is not replicated in archaic (Neanderthal and Denisovan) and ancient (Anzick, Saqqaq, Ust-Ishim, Mal'ta) hunter-gatherer individuals.

Discussion: A complex scenario explains the observed frequencies of the tested markers in hunter-gatherers. Different factors, such as pleotropic alleles, rainforest selective pressures, and population dynamics, may be collectively shaping the observed genetic patterns. We conclude that although TGH seems a plausible hypothesis to explain part of the data, other factors may be important in our tested populations.

KEYWORDS
adaptation, APOE, mode of subsistence, pleiotropy

1 | INTRODUCTION

The first hunter-gatherers who arrived in America, which was the last continent to be colonized by modern humans in prehistoric times (~15,000–21,000 years before present; Bortolini, González-José, Bonatto, & Santos, 2014; González-José, Bortolini, Santos, & Bonatto, 2008), were subjected to a wide range of environmental conditions, including climate, food availability, pathogens, and altitudes. Over time and in some regions, hunter-gatherer/forager societies gave rise to sedentary farming and/or herding communities, which in turn supported the emergence of sophisticated urban civilizations in the pre-Columbian era (Salzano and Bortolini, 2002). In other places, however, small and isolated populations in Amazonia and the Brazilian Central Plateau have maintained their traditional hunter-gatherer survival strategies until today (FUNAI, 2016; Survival International, 2016).

Even acknowledging the role of genetic drift and other stochastic processes in shaping the genetic diversity of Native Americans (Paixão-Côrtes et al., 2011; Tarazona-Santos et al., 2001; Wang et al., 2007), variable selective pressures may partially account for such patterns, including those that have emerged due to new cultural practices (Laland, Odling-Smee, & Myles, 2010). Several studies have been conducted based on this premise, some of which demonstrated the action of natural selection in the autochthonous adaptation in the Americas (Acuña-Alonzo et al., 2010; Hünemeier et al., 2012; Foll, Gaggiotti, Daub, Vatsiou, & Excoffier, 2014; Jacovas et al., 2015), with others showing negative or contradictory results (Bisso-Machado, Ramallo, Tarazona-Santos, Salzano, Bortolini, & Hünemeier, 2013; Bisso-Machado et al., 2016; Demarchi et al., 2005).

Under modern conditions, such as food overabundance, energy homeostasis is unbalanced and lead to the emergence of so-called "diseases of civilization" (e.g., obesity, type 2 diabetes, and related comorbidities). To explain the evolutionary basis of such phenomenon, there are nowadays at least two major and conflicting hypotheses: the thrifty genotype hypothesis (TGH) and the drifty genotype hypothesis. James V. Neel proposed the TGH in 1962 (Neel, 1962) and updated it later with the addition of new knowledge (Neel, Weder, & Julius, 1998). In short, the TGH proposes that in prehistoric times, famines, and seasonal shortages of food were common and severe enough to select thrifty genotypes, which became detrimental when nutritional conditions improved and lifestyles changed. Conversely, Speakman (2008) drifty genotype hypothesis claims that drift, and not selection, is the main driving force to explain current distributions of genetic variants predisposing for obesity in modern populations, after heavy selection by predation was alleviated. Additionally, a third hypothesis was suggested: The thrifty phenotype hypothesis (Hales and Barker, 2001), which proposes an association between poor nutrition in early life and the subsequent development of metabolic syndrome and type 2 diabetes, due to permanent changes in glucose-insulin metabolism in these individuals.

In the context of the TGH, cultural changes (for instance, adoption of an agriculturalist and sedentary lifestyle) can promote new and powerful selective pressures, which can significantly influence the genomes of modern human populations. To expand the studies on the interactions between genes and cultural processes, scientists developed the gene-culture coevolutionary theory, which was described as "a branch of theoretical population genetics that models the transmission of genes and cultural traits from one generation to the next, exploring how they interact" (Feldman and Laland, 1996). More recently, Laland et al. (2010) identified more than 100 genes that have undergone a rapid and recent positive selection due to cultural pressures, particularly due to the adoption of farming and/or herding by modern humans. These genes are involved in immune response, feeding behavior, and several metabolic processes, such as cholesterol metabolism and synthesis. Following the study by Laland et al., many other genes belonging to distinct genetic networks have been associated with the so-called "diseases of civilization."

The first well-documented example of gene-culture coevolution in Native Americans was published recently and was found to be associated with diet (Acuña-Alonzo et al., 2010; Hünemeier et al., 2012). The authors of the study applied Neel's TGH to explain the distribution of the *ABCA1* Arg230Cys alleles, which are associated with cholesterol efflux and high-density lipoproteins (HDL) levels in Native American populations. In the context of TGH, the 230Cys allele could have conferred a protective effect under conditions of nutritional deprivation in Mesoamerica during the transition from hunter-gatherer/forager societies to incipient agriculturalists, who survived on a maize-based diet. However, in contemporary populations the 230Cys is associated with obesity and associated comorbidities (Acuña-Alonzo et al., 2010).

Considering the abovementioned results and that the candidate gene approach is an extremely powerful method (Kim, Lee, Kim, Lee, & Kim, 2012), we investigated selected metabolism-related markers in 375 Native South American volunteers. The selected markers include two *APOE* isoform-determining SNPs (rs429358 and rs7412) and other SNPs located in metabolic genes with known biological functional impact but with absent or limited information in Native American populations, namely, *APOA2* (rs5082), *CD36* (rs3211883), *TCF7L2* (rs11196205), and *IGF2BP2* (rs11705701) (See Materials and Methods). Two major modes of subsistence were incorporated in our analyses, namely, agriculture and hunter-gathering/foraging. Our main goal was to determine whether the patterns of variability observed in the two sets of populations could be associated with their respective diet habits, and to test whether the results are consistent with Neel's hypothesis. The results of the study could provide new examples demonstrating the role of culture practices in shaping the genetic repertoire of Native Americans.

2 | MATERIALS AND METHODS

2.1 | Samples and ethical procedures

Six selected SNPs were genotyped in 375 volunteers from 17 populations who were characterized as Native American or as having high Native American ancestry (~90%; Sandoval et al., 2013, 2016). Genotyped individuals and samples were grouped into two traditional modes of subsistence: (a) Hunter-gatherers/foragers from Brazil: Apalaí

($n = 46$), Gavião ($n = 7$), Guarani ($n = 46$), Jamamadi ($n = 7$), Surui ($n = 10$), Wai-Wai ($n = 8$), and Zoró ($n = 12$); Paraguay: Lengua ($n = 14$); and Peru: Andoas ($n = 57$). Andoas is a district of a Peruvian Amazonian region (Loreto Department). Andoas people belong to the Achuar Confederation (Jivaro linguistic family) and are related to other groups of the same linguistic family, as well as the Amazonian Kichwa (Sandoval et al., 2016). Thus, Andoas are considered as hunter-gatherer/forager Amazonians. The Guarani are the representative group of the people who were part of the extraordinary Tupian migration from the Amazonian region to the South. Therefore, except for the Lengua population, Andoas, Apalai, Gavião, Guarani, Jamamadi, Surui, Wai-Wai, and Zoró can be considered typical populations of the rain forest environment; and (b) Andean agriculturalists and camelid-herding communities from Peru: Amantani ($n = 29$), Anapia ($n = 15$), Cabanaconde ($n = 7$), Chivay ($n = 4$), Taquile ($n = 41$), Yanque ($n = 10$), Quechua ($n = 40$), and Uros ($n = 22$). For comparison and sample enrichment for *APOE* markers, *APOE* genotype data from 39 Aymara individuals from Bolivia listed in Gayà-Vidal et al., (2012) were included in the analyses. Unlike others groups living within the same vicinity, the Uros are not classical Andean agro-pastoralists since they traditionally live in the floating islands of Lake Titicaca. However, Uros were assigned to the Andean agro-pastoralists in the present study, since results of previous genetic studies revealed that they are more closely related to the Aymara and Quechua than to Amazonian people (Sandoval et al., 2013).

Caution is needed regarding modes of subsistence practices, since they may change over time. Additionally, hunter-gatherer/forager communities can also practice some level of incipient agriculture. However, here we consider as agriculturalists those populations that drastically changed their historical trajectory due to adoption of agriculture and/or pastoral activities in the Pre-Colombian era. For instance, these populations became sedentary, thereby promoting the emergence of the urban nucleus (e.g., Inca Empire in South America). However, we considered as hunter-gatherer/foragers those populations that currently (or until recently) follow lifestyles similar to that of their ancestors in the more remote areas of South America.

Ethical approval for our study was provided by the Brazilian National Ethics Commission (CONEP Resolution no. 123/98) and by ethics committees in the countries where non-Brazilian samples were collected. All sampling procedures were performed according to the Helsinki Declaration. The ethics committees also approved the oral consent procedure—since most participants were illiterate—as well as the use of those samples for populational and evolutionary studies.

2.2 | Genes and SNP selection

Many SNPs are involved in metabolic gene networks (See the OMIM website). Thus, there are a large number of potential candidate genes and SNPs that could be analyzed for the indicated purpose. In this study, we selected SNPs with known biological function but little or no data available for Native American populations.

The *APOE* (chr19: 44,905,754-44,909,393/hg38) locus encodes apolipoprotein E, which plays a central role in normal metabolism and is particularly involved in cholesterol and lipid transport and delivery within tissues, neuron repair and maintenance, as well as immune response (Kesäniemi, Ehnholm, & Miettinen, 1987; Mahley, 1988; Mahley and Rall, 2000; Mahley, Weisgraber, & Huang, 2006; Vitek, Brown, & Colton, 2009). The allelic composition of two SNPs rs429358 C/T (R/C at position 130) and rs7412 C/T (R/C at position 176) determine three common haplotypes (E4, E3, and E2) that encode three protein isoforms. The *APOE* polymorphism has been previously studied in Native American populations; however, previous authors did not classify samples according to dietary habits (Andrade et al., 2000; Demarchi et al., 2005).

In addition to known functional impact and scarce or absent genetic information in Native American populations, we selected SNPs that have a Minor Allele Frequency (MAF) of $\geq$1% and are located in exons, introns, or 5′ and 3′ flanking regions, and thus are likely to exert a regulatory function. Subsequently, we restricted the number of SNPs to those with between-group (F_{CT}) component of variance of >4% and considered the following pairwise comparisons among the three major continental groups from which data were available: African versus European, European versus Asian, and Asian versus African. The HAPMART tool of HapMapPhase II database release 21, which was available at the time the research was conducted, was used to analyze the data.

The *APOA2* gene (chr1: 161,222,292-161,223,631/hg38) encodes apolipoprotein A-II, the second most abundant protein in HDLs (Borghini, James, Blatter, & Pometta, 1991) and has been associated with obesity and insulin resistance in several animal and human studies (Corella et al., 2011, and references therein). Studies have shown that the 256A > G promoter polymorphism (rs5082) is associated with lower transcriptional rate and ApoA-II levels, as well as lower waist circumference (Garver, 2011; van't Hooft, Ruotolo, Boquist, de Faire, Eggertsen, & Hamsten, 2001).

CD36 (chr7: 80,369,575-80,679,277/hg38) is a B scavenger membrane receptor that performs a vast array of metabolic functions. *CD36* facilitates the transport of long fatty acids and other large ligands, such as oxidized low-density lipoproteins, into cells, which in turn influences lipid utilization in the muscle, adipose energy storage, and gut fat absorption (Silverstein and Febbraio, 2009). In the clinical context, *CD36* has been linked to platelet glycoprotein IV deficiency, a bleeding disorder that mainly affects Africans and Asians. Some authors have highlighted the role of *CD36* in atherosclerosis (Park, 2014), obesity (Love-Gregory and Abumrad, 2011), and malaria susceptibility (Aitman et al., 2000; Cserti-Gazdewich et al., 2009). Several potential functional SNPs have been identified, including intron rs3211883A > T. However, the prevalence of the risky allele (ancestral-A or derived-T) varies among populations (Bokor et al., 2010; Choquet et al., 2011; Heni et al., 2011).

TCF7L2 (chr10: 112,950,250-113,167,678/hg38), transcription factor 7-like 2, is a component of the Wnt pathway, and variants in this locus have been strongly associated with type 2 diabetes mellitus (T2D) across different world populations and ethnic groups (Grant et al., 2006; Yao et al., 2015). Miyake et al. (2008) and Ng et al. (2007) studied the intronic polymorphism rs11196205 C > G and suggested

an association between the ancestral allele C and higher risk of T2D in Hong Kong Chinese and Japanese patients.

Lastly, *IGF2BP2* (insulin-like growth factor 2 mRNA-binding protein; chr3:185,643,739-185,825,056/hg38) belongs to a family of binding proteins, and its physiological role remains to be elucidated. However, *IGF2BP2* is fundamentally expressed during embryo development and is known to regulate the translation of insulin-like growth factor 2 (IGF2). Results of GWAS studies showed that rs11705701 A > G is positively associated with T2D (with A being the risk allele) and percentage of body fat (Chistiakov et al., 2012, and references therein). Notably, Li et al. (2009) showed that the interaction between rs11705701 genotype and body fat influences insulin resistance and contributes to T2D risk in Mexican Americans.

2.3 | Laboratory methods

DNA was extracted from plasma, glycerolized red blood cells, and whole blood stored in our laboratories from previous studies conducted by our research group (review in Bisso-Machado et al., 2015; Jacovas et al., 2015; Salzano, 2002) using the QIAamp DNA MiniKit (Qiagen, Germany). Samples from non-Brazilian populations were provided by co-authors of the present study (JRS and AS-G). SNP genotyping was performed using the TaqMan Genotyping Assays (Applied Biosystems; Supporting Information Table S1) following the manufacturer's instructions, in the ECO®RealTime PCR System (Illumina). In cases where the TaqMan genotype data were insufficient to obtain and/or confirm the results, sequencing was outsourced to an external service provider. Unfortunately, TaqMan SNP Genotyping assays did not work for some DNA samples, since they were collected a long time ago, and DNA was degraded or was of low quality.

2.4 | Statistical analyses

Adjustment to the Hardy-Weinberg equilibrium was assessed using the chi-square test. Analyses of molecular variance (AMOVA) were performed in Arlequin v3.5.2.1 (Excoffier and Lischer, 2010) to evaluate the variance among and within the investigated Native American populations.

Allele distributions were tested for association with modes of subsistence employing Fisher's Exact test in PLINK v.1.07 (Purcell et al., 2007) using the hunter-gatherer and agriculturalist categories as outcomes and SNPs as predictors. *APOE* isoform frequencies were compared using Exact Fisher's test in PASW v.18.0 (SPSS, 2009). Post-hoc examination of each cell was done by interpretation of adjusted standardized residuals. For SNPs with MAFs higher than 10% in the whole sample, SNP-SNP interactions were evaluated via logistic regression using an additive scale with PASW v.18.0 and performed as described in Andersson, Alfredsson, Källberg, Zdravkovic, & Ahlbom, (2005). This approach also allows the estimation of measures of interaction, such as the attributable proportion (AP), and the synergy index (SI). AP is the proportion of the combined effect that is explained by the interaction, while SI is the ratio between the combined effect and individual effects. When AP = 0 and SI = 1 there is no interaction effect beyond what is expected in exact additivity. However, if AP > 0 and SI > 1 there are positive interaction effects that exceed what would be expected under the additivity model (Knol et al., 2011).

2.5 | Paleoanthropological dataset

Genetic, archeological, and linguistic evidence indicate that the first Americans descended mostly from ancestral Siberians (Goldberg, Mychajliw, & Hadly, 2016; González-José Bortolini, Santos, & Bonatto, 2008; Santos et al., 1999). Thus, data for the same previously indicated SNPs were compiled from four Siberian and Native American hunter-gathering ancient individuals whose genomes are known (Raghavan et al., 2014; Rasmussen et al., 2010, 2014). Additionally, genotype data from two distinct ancient humans, namely, one Siberian Neanderthal and one related Denisovan who lived in the same region, were also compiled (Prüfer et al., 2014; Reich et al., 2010; Supporting Information Table S2).

3 | RESULTS

3.1 | Population diversity and structure analyses

AMOVA results and allele frequencies for each population and locus are presented in Table 1. Most populations in both groups are polymorphic for all loci investigated. The ancient Native and Siberian individuals (Supporting Information Table S2) show genotypes compatible with contemporary individuals who inhabit their corresponding regions. Furthermore, the presence of individuals heterozygous for *IGF2BP2* rs11705701 (Saqqaq and Ust'-Ishim), and *APOE* rs429358 (Anzick, Saqqaq, and Ust'-Ishim) suggests a certain degree of population variability, since multiple alleles are segregating for both loci.

Regarding *APOE* haplotypes (Table 2), all known isoforms were detected in the hunter-gatherer group, with E3 being the most common. Interestingly, three genotypes (E2/E2, E2/E3, and E2/E4) carrying the less-frequent E2 allele were also found. The agriculturalist group showed lower variation, since only three genotypes were detected, with E3/E3 being the most frequent. No homozygotes for E2 were found, but the number of populations investigated is lower for this group.

AMOVA was independently performed for each locus to determine genetic structure among and within subsistence groups (Table 1). For F_{ST}, only one SNP (rs3211883, *CD36*) yielded significant values in both agriculturalist and hunter-gatherer groups. For F_{CT}, only *APOE* rs429358 yielded significant values ($p = 0.00196$), suggesting strong differences in the allelic distribution between hunter-gatherers and agriculturalists. The same pattern was not observed in *APOE* rs7412, but this result could be explained by the low sample size for the agriculturalists ($n = 79$), although a trend in the same direction was observed ($p = 0.06843$).

Analyses based on isoform frequencies and AMOVA were also performed on individuals with available genotype data for both *APOE* rs429358 and *APOE* rs7412 (Table 2). Since only one individual remained in the Andoas population after data filtering, this population

TABLE 1 Allele frequencies and AMOVA results for Native South American populations classified based on two major modes of subsistence (agriculturalists and hunter-gatherers)[a]

Populations (Total N)	*APOE* rs429358 n	T	C	rs7412 n	T	C	*APOA2* rs5082 n	A	G	*CD36* rs3211883 n	A	T	*TCF7L2* rs11196205 n	C	G	*IGF2BP2* rs11705701 n	A	G
Agriculturalists																		
Amantani (29)	11	0.909	0.091	0	NA	NA	12	1.000	0.000	28	0.321	0.679	26	0.000	1.000	29	0.086	0.914
Anapia (15)	3	1.000	0.000	0	NA	NA	0	NA	NA	15	0.600	0.400	15	0.067	0.933	15	0.033	0.967
Chivay (4)	3	1.000	0.000	0	NA	NA	0	NA	NA	4	0.750	0.250	3	0.000	1.000	3	0.333	0.667
Cabanaconde (7)	4	1.000	0.000	0	NA	NA	7	1.000	0.000	1	0.000	1.000	4	0.000	1.000	4	0.000	1.000
Quechua (40)	40	0.963	0.038	40	0.000	1.000	28	0.964	0.036	35	0.371	0.629	40	0.013	0.988	40	0.175	0.825
Taquile (41)	7	1.000	0.000	0	NA	NA	0	NA	NA	41	0.098	0.902	41	0.000	1.000	41	0.305	0.695
Uros (22)	6	1.000	0.000	0	NA	NA	19	1.000	0.000	22	0.341	0.659	22	0.000	1.000	22	0.227	0.773
Yanque (10)	6	0.833	0.167	0	NA	NA	6	0.833	0.167	10	0.400	0.600	10	0.000	1.000	10	0.100	0.900
Aymara* (39)	39	0.949	0.051	39	0.013	0.987	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
F_{ST} (*p*-value)	−0.05618 (0.91691 ± 0.00628)			0.00000 (1.00000 ± 0.00000)			0.02707 (0.30205 ± 0.01574)			**0.10409 (0.00098 ± 0.00098)**			−0.01264 (0.51417 ± 0.01484)			0.04766 (0.06061 ± 0.00730)		
Hunter-gatherer/foragers																		
Andoas (57)	6	0.583	0.417	1	0.000	1.000	0	NA	NA	57	0.211	0.789	57	0.035	0.965	57	0.184	0.816
Apalai (46)	46	0.870	0.130	45	0.044	0.956	35	0.943	0.057	42	0.190	0.810	42	0.048	0.952	44	0.227	0.773
Gavião (7)	5	0.600	0.400	6	0.167	0.833	1	1.000	0.000	4	0.250	0.750	7	0.000	1.000	7	0.286	0.714
Guarani (46)	45	0.811	0.189	46	0.065	0.935	14	0.893	0.107	35	0.457	0.543	45	0.111	0.889	46	0.228	0.772
Jamamadi (7)	7	0.714	0.286	6	0.000	1.000	1	1.000	0.000	4	0.000	1.000	6	0.167	0.833	7	0.357	0.643
Lengua (14)	14	0.857	0.143	13	0.000	1.000	7	0.929	0.071	12	0.583	0.417	14	0.000	1.000	14	0.000	1.000
Surui (10)	8	0.813	0.188	8	0.000	1.000	9	1.000	0.000	9	0.389	0.611	9	0.000	1.000	9	0.056	0.944
Wai-Wai (8)	8	0.438	0.563	8	0.000	1.000	7	1.000	0.000	8	0.313	0.688	8	0.000	1.000	8	0.188	0.813
Zoró (12)	11	0.818	0.182	10	0.000	1.000	10	0.950	0.050	9	0.444	0.556	9	0.000	1.000	10	0.050	0.950
F_{ST} (*p*-value)	0.03480 (0.14467 ± 0.01239)			−0.02074 (0.65885 ± 0.01453)			−0.08177 (0.96481 ± 0.00704)			**0.06196 (0.03421 ± 0.00608)**			−0.00071 (0.39883 ± 0.01392)			−0.01023 (0.61095 ± 0.01886)		
F_{CT} (*p*-value)	**0.10819 (0.00196 ± 0.00136)**			0.02473 (0.06843 ± 0.00643)			0.00873 (0.20626 ± 0.01415)			0.02163 (0.86217 ± 0.01390)			0.01751 (0.11241 ± 0.00902)			−0.00757 (0.76735 ± 0.01244)		

[a]Significant values in bold. F_{ST} and F_{CT} are among-population and between-group components of variance, respectively. NA: Not available. * Retrieved from Gayà-Vidal et al. 2012.

TABLE 2 *APOE* genotype and haplotype frequencies and AMOVA results for Native South American populations classified according to two major modes of subsistence[a]

	Genotypes							Allele frequencies		
Populations	*N*	E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	E2 (rs429358 T, rs7412 T)	E3 (rs429358 T, rs7412 C)	E4 (rs429358 C, rs7412 C)
Hunter-gatherers/foragers										
Apalai	45	2	0	0	31	12	0	0.0444	0.8222	0.1333
Gavião	5	1	0	0	1	2	1	0.2000	0.4000	0.4000
Guarani	45	0	5	1	25	12	2	0.0667	0.7444	0.1889
Jamamadi	6	0	0	0	3	2	1	0.0000	0.6667	0.3333
Lengua	13	0	0	0	10	3	0	0.0000	0.8846	0.1154
Surui	7	0	0	0	5	2	0	0.0000	0.8571	0.1429
Wai-Wai	8	0	0	0	1	5	2	0.0000	0.4375	0.5625
Zoró	10	0	0	0	7	2	1	0.0000	0.8000	0.2000
F_{ST} (*p*-value)	0.02743 (0.14858 ± 0.01350)									
Agriculturalists										
Quechua	40	0	0	0	38	2	0	0.0000	0.9750	0.0250
Aymara	39	0	0	1	35	3	0	0.0128	0.9359	0.0513
F_{ST} (*p*-value)	−0.01632 (0.62561 ± 0.01568)									
F_{CT} (*p*-value)	0.09763 (0.11828 ± 0.00836)									

[a]F_{ST} and F_{CT} represent among-population and between-group components of variance, respectively. Values were calculated using haplotype frequencies.

was excluded from the analysis. *APOE* E2, E3, and E4 isoforms showed similar AMOVA results for individual SNPs with nonsignificant interpopulation F_{ST} and between subsistence groups F_{CT} values. However, the frequency of the E4 haplotype in the hunter-gatherer group (from 0.1154 in Lengua to 0.5625 in Wai-Wai) is evidently higher than those in the agriculturalists (0.0250 in Quechua and 0.0513 in Aymara).

3.2 | Main effects and gene-gene interaction analyses

Association analyses were carried out using mode of subsistence as outcome. A significant effect was found for *TCF7L2* rs11196205, in which the ancestral C allele showed higher frequencies in the hunter-gatherer group ($p = 2.1 \times 10^{-3}$, OR = 5.69, Supporting Information Table S3). Similarly, the *APOE* rs429358C and *APOE* rs7412T ancestral and derived alleles, respectively, were more frequently observed in the hunter-gatherer group than the agriculturalist group ($p = 2.2 \times 10^{-8}$, OR = 5.38 and $p = 0.0393$, OR = 6.88, respectively). Residual analysis for *APOE* isoforms showed that the E3/E3 genotype was significantly more widespread in the agriculturalist group, whereas E4 allele carriers were more frequent in the hunter-gatherer group ($p = 1.2 \times 10^{-5}$, Supporting Information Table S4), thereby supporting the association between the rs429358/E4 allele and hunter-gatherers.

Gene-gene interactions were evaluated using logistic regression analysis to test whether the associations found for the *APOE* isoforms were modulated by other SNPs. *APOA2* rs5082 and *TCF7L2* rs11196205 were not tested due to low MAFs. Individuals were classified as either carriers or noncarriers of a given variant (e.g., E3 homozygotes vs. E4 allele carriers) to increase statistical power. Figure 1a shows a deviation from exact additivity when *APOE* isoforms and *IGF2BP2* rs11705701 are jointly considered (AP = 0.421; SI = 1.97). *APOE* E4/*IGF2BP2* GG individuals were significantly more frequent in the hunter-gatherer group (OR = 4.28; $p = 0.026$) when compared to the reference category (*APOE* E3E3/*IGF2BP2* GG) probably due to the main effect that *APOE* E4 showed in the single marker analyses. It should be stressed that there was an important trend showing that the *APOE* E4/*IGF2BP2* A carriers are even more frequent in the hunter-gatherer group (OR = 7.03; $p = 0.065$) as compared to the reference category, highlighting potential synergism effects. A positive interaction was found between *APOE* isoforms and *CD36* rs3211883 (AP = 0.422; SI = 1.86). *APOE* E4/*CD36* A carriers were significantly more frequent in the hunter-gatherer group (OR = 6.24; $p = 0.020$) when compared with the reference category (*APOE* E3E3/*CD36* A carrier). *APOE* E4/*CD36* TT individuals were even more frequent in the hunter-gatherer group (OR = 11.40; $p = 0.022$), as compared with the reference category.

4 | DISCUSSION

Native American populations represent an attractive subject for population analyses, considering their unique demographic, evolutionary, and cultural histories. However, despite the research interest and

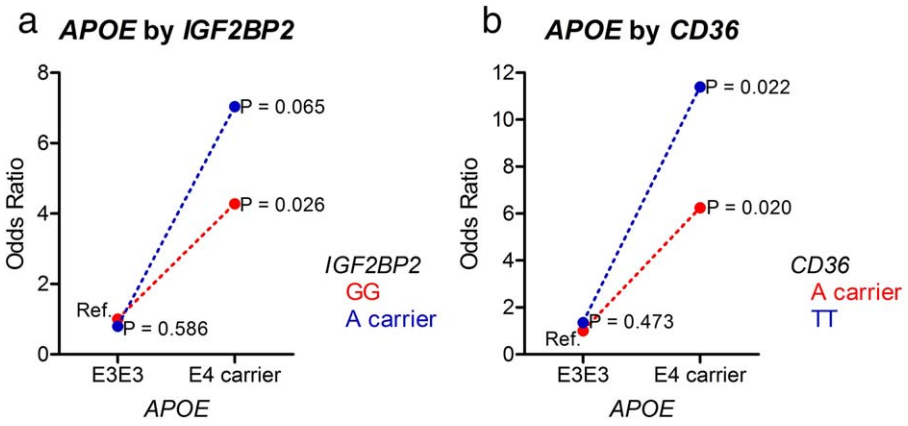

FIGURE 1 Interaction effects on mode of subsistence. In the logistic regression model agriculturalists were coded as 0 and hunters-gatherers as 1. A) *APOE* E3E3 individuals were coded as 0 and E4 allele carriers as 1. *IGF2BP2* GG individuals were coded as 0 and A allele carriers as 1. AP = 0.421 and SI = 1.965. Reference category (Ref.) = *APOE* E3E3/IGF2BP2 GG. B) *APOE* E3E3 individuals were coded as 0 and E4 allele carriers as 1. AP = 0.422 and SI = 1.859. Due to the protective OR described for *CD36* rs3211883 in table S3, we recoded A allele carriers as 0 and TT individuals as 1. Reference category (Ref.) = *APOE* E3E3/ CD36 A carrier.

efforts to study them in the past years, genetic knowledge on Native Americans is still limited compared to other major continental groups. This is mainly due to low accessibility, since most populations live in geographically remote areas of South America. In addition, until today, these small and isolated communities survive mainly by using hunter-gatherer strategies, speaking rare native languages, and in recent times strongly reacted to past ways they were treated by researchers. All these factors substantially limit comprehensive investigations. Thus, obtaining research material from these populations remains a challenging task (Salzano, 2015). This study intends to help fill the gap by providing novel genetic data from populations with distinct modes of subsistence, based on the hypothesis that cultural practices leave marks in their metabolic genetic background by exerting selective pressures on populations (Laland et al., 2010).

In the revision of the TGH in 1998, Neel et al. suggested that the processes that result in genetic predisposition to civilization-related conditions may be more complex than previously stated; nonetheless, TGH remains viable within the broader context of civilization-related diseases. TGH has been intensively discussed and challenged in more recent years at both the theoretical (Speakman, 2008) and empirical (Gosling, Buckley, Matisoo-Smith, & Merriman,, 2015) levels. J. R. Speakman focused on obesity to evaluate Neel's TGH, arguing that periods of famine are not sufficient to explain the prevalence of obesity in western societies, and proposing a number of alternatives, including the drifty genotype hypothesis (Speakman, 2008, 2013). However, TGH may not only apply to obesity. Other not so evident phenotypic traits, which may cause problems in the course of an individual's life (e.g., atherosclerosis), may be influenced by genetic variants which, in a given period or environment, may be advantageous for survival and fertility, and later detrimental. This idea has also been discussed elsewhere (Corbo & Scacchi, 1999; Corbo, Scacchi, & Cresta,, 2004; Reales et al., 2014). Gosling et al. (2015) challenged TGH as an explanation for metabolic diseases in Pacific populations, suggesting alternative factors (e.g., disease epidemics and social/cultural selection for particular phenotypes) as responsible for the current genetic landscape in Oceania.

However, a genome-wide study in Samoans identified an Arg > Gln variant at position 457 of *CREBRF* that is associated with a 1.3-fold increased risk for obesity. Samoans exhibit one of the highest obesity rates in the world. This variant, which has high frequencies in Samoans (26%) but is extremely rare in other populations, was positively selected in the past since it promotes fat storage and reduces energy use in cells, which are requirement conditions for the TGH (Minster et al., 2016). More recently, Amorim et al. (2017) detected a robust positive selection signal for fatty acid desaturases (*FADS*) genes throughout the Americas. The shared signature of selection among Native Americans living in diverse environments such as Arctic and Amazonia is likely due to a single and strong instance of local adaptation to the cold climate and to a protein-rich diet that took place in Beringia, before their entrance into the New World. The impact of this genetic background for Native Americans living in modern changing environments, and its implication for the TGH, deserves to be investigated. We observed significantly higher frequencies for the thrifty allele in hunter-gatherers (rs429358C, *APOE*), but no significant differences in most of the other markers. Due to its vital role in various processes, the profound influences of *APOE* on human health and lifespan have been broadly studied (Joshi, Fischer, Schraut, Campbell, Esko, & Wilson, 2016; Napolioni, Giannì, Carpi, Predazzi, & Lucarini, 2011; Schupf et al., 2013; Trotter, Liebl, Weeber, & Martin, 2011). E2 carriers have lower plasma levels of total and LDL cholesterol than E3 carriers. However, E4 displays an opposite pattern and is associated with major risk for several contemporary conditions, including Alzheimer's disease, atherosclerosis, coronary artery diseases, and other related conditions (Corbo and Scacchi, 1999; Fullerton et al., 2000; Reales et al., 2014 and references therein). The E2, E3, and E4 haplotypes show varying distributions among human populations, with E3 being the most prevalent in many human groups (Singh, Singh,& Mastana, 2006). E4, the

ancestral allele, is fixed in chimpanzees and other primates (Hanlon and Rubinsztein, 1995), whereas E3 is estimated to have appeared within the last 235,000 years (Trotter et al., 2011). Denisova is homozygous for the E3 haplotype, which implies that E3 was segregating in *Homo* populations 550-765 kya before splitting of the archaic/African lineage (Denisova + Neanderthal/*H. sapiens*; Prüfer et al., 2014).

The E2/rs7412T allele is rare in the examined populations, being found only in three of our populations (Apalai, Gavião, and Guarani), the Aymara population (Gayà-Vidal et al., 2012), and one Wai-Wai individual from a previous study (Andrade et al., 2000). The E2/rs7412T allele is present in 10% of Eastern Asians (1000 Genomes Project Consortium, 2015), which is the continental group geographically closest to Native Americans and could represent the source of the E2 allele in the populations analyzed in this study. However, rs7412T was not detected in ancient Native American individuals (i.e., Saqqaq and Anzick); thus, a recent admixture event with non-Indians cannot be ruled out. However, the absence of rs7412T in the Neanderthal and Denisovan groups suggests that this variant emerged in the *Homo sapiens* lineage at less than 550–765 thousand years ago (Endicott, Ho, & Stringer, 2010; Prüfer et al., 2014).

E4 remains prevalent in some hunter-gatherer/forager populations and was thus also identified as a thrifty allele (Corbo and Scacchi, 1999). Other hypotheses explaining *APOE* allele distributions have been proposed, including the "grandmother" and the meat-adaptive gene hypotheses, immunological factors (Trotter et al., 2011), and nutrition factors (Egert, Rimbach, & Huebbe, 2012).

Our data reinforce the functional importance of *APOE* E4, considering its high prevalence in Native American populations who are characterized as hunter-gatherers. The E4 allele would confer significant advantage in more severe conditions such as food scarcity and exposure to various pathogens and parasites, as is normally found in hunter-gatherer societies. Trotter et al. (2011) suggested that *APOE* alleles were positively selected due to their differential susceptibility to diverse parasites, since E4 is associated with a pro-inflammatory phenotype. This allele has also high prevalence in hunter-gatherer societies from Africa (Pygmies, 41%; Khoisan, 37%) and Australasia (Malaysian aborigines, 24%; Australian aborigines, 26%; Papuans, 37%; Trotter et al., 2011); therefore, we suggest that the E4 allele confers evolutionary advantage in environments subjected to strong selective pressures for immunological response and energy homeostasis.

Carriers of *APOE* E4-*CD36*TT and *APOE* E4-*IGF2BP2* rs11705701A are more frequently observed in the hunter-gatherer group, possibly indicating preference for combinations of thrifty alleles. The *IGF2BP2* rs11705701A allele was positively correlated with substantially higher risk of developing T2D, whereas *CD36*TT carriers, at least in some populations, showed significantly larger waist circumferences and body mass indices (Heni et al., 2011). Interestingly, this genetic combination (ancestral and derived alleles) also shows a positive synergistic effect, which supports their possible effect on energy homeostasis. These arrangements would be selected in environmental conditions of low caloric intake, as the rainforest (Shea and Bailey, 1996). Notably, with the exception of the Lengua (from the Chaco), all hunter-gatherer populations of our dataset can be considered as typical Amazonian rainforest dwellers, with the Guarani living today in the Central-Southern region of South America due to their relatively recent migration from the Amazonian region to the South (Marrero et al., 2007).

CD36 was one of the two loci where interpopulation heterogeneity was detected based on F_{ST} (Table 1). The role of ancestral and derived rs3211883 alleles in susceptibility to civilization-related diseases and related phenomena remains controversial and depends on the population being investigated (Bokor et al., 2010; Choquet et al., 2011; Heni et al., 2011). Despite the influence of known methodological confounding factors in case-control studies, these contradictory results may indicate that other important modulating factors may influence allele frequency outcomes in each population. In this context, a certain degree of structure within the hunter-gatherer group is expected in the *CD36* rs3211883 allele distribution.

Furthermore, *CD36* encodes the platelet glycoprotein 4, an adhesion molecule involved in the interaction between parasite ligands and host receptors. Most *P. falciparum* antigens are known to bind *CD36*; thus, mutations in the *CD36* gene can influence malaria outcome (Aitman et al., 2000; Cserti-Gazdewich et al., 2009; Kajeguka et al., 2012). The higher frequency of *CD36*TT in hunter-gatherer populations, therefore, may be also due to immunologic selective pressure, a possibility not mutually exclusive with TGH, since *CD36*TT genotype was also associated with larger waist circumferences and body mass indices in some populations (Heni et al., 2011).

The hunter-gatherer lifestyle is characterized by a steady scarcity of resources and heavy energy investment in obtaining food. Thus, constant selective pressures associated with this mode of subsistence may have resulted in the conservation of general thrifty markers among them, with some degree of interpopulation diversity, according to their specific genetic repertoire, ecological demands, and selective pressures. Remarkably, archaic (Neanderthal and Denisovan) and ancient hunter-gatherers (Anzick, Saqqaq, Ust-Ishim, and Malta), present heterogeneous allele combinations for the loci investigated in this study (Supporting Information Table S2), thereby reinforcing the idea that a thrifty genotype in a specific population or geographical group is not necessarily found in others.

However, plant cultivation and animal domestication under favorable conditions guarantee a constant supply of food. Multiple food sources can also prevent food shortages and long periods of hunger. However, this abundant and stable food scene was not observed in many pre-Columbian incipient agriculturalist societies. For instance, Mesoamericans established their sedentary lifestyle based mainly on a single plant (maize), and may have experienced varying periods of scarcity and famine that could have left thrifty genetic footprints (Acuña-Alonzo et al., 2010; Hünemeier et al., 2012). Conversely, a similar scenario has not been described for Andean native populations so far (Gayà-Vidal et al., 2012). In pre-Columbian times, Native Andean agriculturalists cultivated a diverse range of crops and domesticated mammals (e.g., llama and guinea pig), which may have prevented periods of food shortages during the transition from the hunter-gatherer to a sedentary lifestyle.

So far, most studies on hunter-gatherer Native American populations suggest factors other than selection to explain the observed allele frequencies in most markers studied (e.g., *APOE*, Andrade et al., 2000), *PAX9* (Paixão-Côrtes et al., 2011), and *NAT2* (Bisso-Machado et al., 2016, among others). These factors include founder effects, isolation, and genetic drift. However, we would expect hunter-gatherer populations to be also subject to strong selective pressures, including those promoted by cultural practices (Feldman and Laland, 1996). Nonsignificant results for positive selection in the abovementioned studies may be explained by the small sample sizes, as well as limited data availability on the histories of some tribes, which prevents the detection of significant genetic variations. In addition, for some populations, specific alleles might have been positively selected, while drift has been more relevant in others. Different forces may also act simultaneously, shaping the genetic landscape of the population, rendering the discovery of a unifying theory to explain these processes more difficult. Therefore, we acknowledge that TGH is not the only plausible explanation for the higher prevalence of metabolic diseases observed in contemporary populations; rather, our data suggest a complex scenario in which several factors might be acting.

In summary, we describe two genetic combinations, namely, *APOE* E4 × *CD36* TT and *APOE* E4 × *IGF2BP2* A that were observed at higher frequencies in the hunter-gatherer group, and could thus represent an essential part of the genetic repertoire that allowed a gain in fitness in these populations. Finally, we suggest that TGH can only be understood if the multiple mechanisms by which selection pressure acts in different and changing cultural and environmental contexts are considered, as well as the genetic repertoire available for selection.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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