Dup-24 bp in the *CHIT1* Gene in Six Mexican Amerindian Populations

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Abstract Chitotriosidase (CHIT, EC 3.2.1.14) is an enzyme secreted by activated macrophages with the ability to hydrolyze the chitin of pathogens. The high activity of this enzyme has been used as a secondary biomarker of response to treatment in patients with Gaucher disease (OMIM 230800). Within the world's population, approximately 6% is homozygous and 35% is heterozygous for the most common polymorphism in the *CHIT1* gene, a 24-bp duplication (dup-24 bp), with homozygosity of this duplication causing inactivation of the enzyme but without major consequences for health. To determine the frequency of the dup-24 bp *CHIT1* gene in indigenous populations from Mexico, 692 samples were analyzed: Purepecha (49), Tarahumara (97), Huichol (97), Mayan (139), Tenek (97), and Nahua (213). We found that the groups were in Hardy-Weinberg equilibrium. The dup-24 bp allele frequency was found to be (in order of highest to lowest) 37% (Mayan),

34% (Huichol and Nahua), 33% (Purepecha), 31% (Tenek), and 29% (Tarahumara).

Introduction

Chitinases comprise a family of enzymes that hydrolyze chitin and have been found in a wide variety of organisms such as plants, nematodes, bacteria, fungi, insects, and mammals (Boot et al. 1995). Human chitinase (CHIT, EC 3.2.1.14) is a 50-kDa enzyme containing a C-terminal chitin-binding domain and is secreted from activated macrophages. This enzyme is proteolytically processed to a C-terminally truncated 39-kDa isoform characterized by hydrolase activity and is primarily thought to play a role in the body's defense against chitin-containing pathogens (Renkema et al. 1997; Boot et al. 1999). CHIT activity has been used in the diagnostic screening of several lysosomal storage diseases, in which this enzyme is usually highly elevated, making this enzyme a useful secondary biomarker for the treatment monitoring of Gaucher disease (OMIM 230800), Niemann-Pick disease (OMIM 257200), Krabbe disease (OMIM 245200), and thalassemia (OMIM 603141) (Guo et al. 1995; Boot et al. 1999).

The *CHIT1* gene (OMIM 600031.0001), localized on chromosome 1q32.1, consists of 11 exons and spans approximately 14 kb of genomic DNA coding a protein of 466 amino acids [UCSC Genomic coordinates (GRCh37): 1:203,185,206–203,198,859]. CHIT enzyme deficiency is recessively inherited, with widely different prevalence rates in various world populations (Boot et al. 1999). Currently, five genomic polymorphisms have been associated with

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diminished CHIT activity: p.G102S (rs2297950), p.G354R (rs9943208), p.A442G/V (rs1065761), a 4-bp deletion across the exon/intron-10 boundary (complex E/I -10) (rs9943208), and a 24-bp duplication (dup-24 bp) (rs3831317) in exon 10 (Lee et al. 2007; Arndt et al. 2013).

The use of CHIT as a secondary biomarker to monitor enzymatic replacement therapy (ERT) in patients with Gaucher disease is common worldwide; however, ruling out the presence of null activity of CHIT is mandatory. The dup-24 bp polymorphism is possibly the most frequent explanation for a deficiency in chitotriosidase activity, resulting from the aberrant splicing and deletion of amino acids 344–372 (Boot et al. 1999). The biologic significance of the very high frequency of this gene polymorphism remains unexplained, which suggests it may have a selective advantage not yet completely understood (Canudas et al. 2001; Lee et al. 2007). In the African population, the dup-24 bp homozygous state is rare, with an estimated frequency of 2% (Malaguarnera et al. 2003). In contrast, the frequency of the homozygous status is approximately 6% in Caucasians (Boot et al. 1999). In Asian groups, the highest frequencies of this polymorphism have been found in South China, followed by South Korea, Southeast Asia, Japan, and Taiwan, with homozygosity frequencies ranging from 45% to 64% (Lee et al. 2007). Regarding Latin American groups, Peruvian and Mexican populations have been studied. In Peruvian populations, the published homozygosity frequencies are 12% (Peruvian mestizo), 22% (indigenous Amerindian), 26% (Amazonian Amerindian), and 12% (Quechua indigenous) (Manno et al. 2014). In Mexican mestizos, the frequency of dup-24 bp has been estimated as 5.56% for homozygosity (Juárez-Rendón et al. 2012), with no known frequencies for Mexican Amerindian groups. Given that higher frequencies of the dup-24 bp polymorphism are observed in Asian populations and other Amerindian populations, in this study, we wanted to investigate the frequency of the dup-24 bp polymorphism in six Mexican Amerindian populations.

Material and Methods

A total of 692 samples of random individuals belonging to six indigenous groups distributed within the Mexican political boundaries were collected for genetic analysis. The samples were composed of Purepecha ($n = 49$), Tarahumara ($n = 97$), Huichol ($n = 97$), Mayan ($n = 139$), Tenek ($n = 97$), and Nahua ($n = 213$). Informed consent was obtained for these analyses, and peripheral blood samples were obtained from the participants after the objectives of this study were discussed with every participant.

The Institutional Review Board of our center approved the scientific and ethics procedures in this study.

DNA was extracted according to standard protocols from peripheral blood leucocytes (Miller et al. 1988). The molecular analysis for dup-24 bp in the *CHIT1* gene was carried out by endpoint polymerase chain reaction (PCR) (Sanguinetti et al. 1994; Juárez-Rendón et al. 2012).

Statistical Analysis

Genotype and allele frequencies were determined by direct counting. Hardy-Weinberg equilibrium was calculated using the chi-square test. The allelic frequencies were compared with the data from eight reported population: Mexican mestizos, Peruvian mestizos, three Peruvian Amerindian populations, Asians, Europeans, and Africans. The distribution of the allelic frequencies between pairs of populations was analyzed with exact test of population differentiation, which is analogous to Fisher's exact test. One million of steps in Markov chain were used to obtain the p -value and a value of $p < 0.05$ being considered significant. Arlequin ver. 3.0 software was used for the statistical analysis (Laurent Excoffier et al. 2005).

Results

We studied the dup-24 bp polymorphism of the *CHIT1* gene in 692 Mexican Amerindians from north (Tarahumara), west (Huichol, Purepecha, Nahua, and Tenek), and south (Mayan) areas of Mexico. In these indigenous groups, we found 308 (44.5%) individuals with a homozygous wild-type genotype (wt/wt), 306 (44.2%) individuals with a heterozygous genotype (wt/dup), and 78 (11.3%) individuals who were homozygous (dup/dup) for the 24-bp duplication. The frequency of dup/dup was observed in a range of 10% (Tarahumara and Nahua) to 14% (Purepecha). In contrast, the allele frequency of dup-24 bp was found to be highest in Mayan (37%) and lowest in Tarahumara (29%) (Table 1). The observed distribution of genotypes fell within the Hardy-Weinberg equilibrium.

In the distribution of the polymorphism genotype frequencies, the six Mexican indigenous groups showed no significant differences among them ($p < 0.05$). However, the Huichol, Mayan, and Nahua showed significant differences with the Mexican mestizo population. These differences are due mainly to an increase in the frequency of the heterozygous wt/dup genotype. Amerindians and Quechua indigenous groups did not differ with the Mexican indigenous populations, and Peruvian mestizos were similar to the Purepecha and Mayan populations.

Table 1 Genotypic and allelic distribution of the dup-24 bp polymorphism in the *CHIT1* gene in Mexican Amerindian populations

Populations	Number	Genotype frequencies (%)						Allele frequencies				Hardy-Weinberg <i>p</i> -value
		Wt/wt	%	Wt/Dup	%	Dup/Dup	%	Wt	%	Dup	%	
Purepechas	49	24	49	18	37	7	14	66	67	32	33	0.2487
Tarahumaras	97	50	52	37	38	10	10	137	71	57	29	0.4261
Huicholes	97	43	45	42	43	12	12	128	66	66	34	0.7265
Mayas	139	55	39	66	48	18	13	176	63	102	37	0.7948
Tenek	97	47	48	40	41	10	11	134	69	60	31	0.7316
Nahua	213	89	42	103	48	21	10	281	66	145	34	0.2618
Mestizos ⁹	306	177	58	112	37	17	6	466	76	146	24	0.8961

Wild type (wt); duplication 24 bp (Dup)

In contrast, the Amazonian Indians, Asians, Europeans, and Africans were different from all of the Mexican populations (Table 2).

Discussion

The use of CHIT as a secondary biomarker has been considered a useful tool for monitoring ERT on patients with Gaucher disease. However, one important caveat is the presence of individuals homozygous for dup-24 bp in *CHIT1* who will require other biomarkers to follow up on their response to ERT (Guo et al. 1995; Lee et al. 2007; Manno et al. 2014). The identification of this polymorphism should also be useful for establishing genetic relationships between populations.

The distribution of this polymorphism was homogeneous across the Mexican Amerindian populations studied, and the Mexican mestizo population was similar to the Purepecha, Tarahumara, and Tenek. We also found a high allelic frequency of the dup-24 bp polymorphism of the *CHIT1* gene, the highest reported for ethnic groups living in Mexico and one of the highest among world populations (Lee et al. 2007). This observation is in accordance with the primarily Asian origin of the American indigenous groups more than 10,000 years ago (Reich et al. 2012) and supports the hypothesis of a selective advantage associated with this gene variant (Lee et al. 2007) or a possible founder effect as well as other markers (Manno et al. 2014). Although the null clinical significance of the dup-24 bp polymorphism in the *CHIT1* gene has been established for some time now (Boot et al. 1998; Canudas et al. 2001; Piras et al. 2007), it has not been determined if there is a clinical significance of the high frequency of homozygosity of dup-24 bp in *CHIT1* in Amerindian populations. Some authors have proposed that a CHIT deficiency can increase the

susceptibility to infections due to parasites containing chitin (Malaguamera et al. 2003), but this implication has to be tested in a future study.

Compliance with Ethics Guidelines

Conflicts of Interest

Thiago Donizete Da Silva-José, Karina Janett Juárez-Rendón, Jesús Alejandro Juárez-Osuna, Angela Porras-Dorantes, Adán Valladares-Salgado, Miguel Cruz, Miriam Gonzalez-Ibarra, Ana G. Soto, María Teresa Magaña-Torres, Lucila Sandoval-Ramírez, and José Elías García-Ortiz declare that they have no conflict of interest.

Originality

The authors state that this is an original article and has not been simultaneously presented to any other journal and thus is exclusive to *Journal of Inherited Metabolic Disease*.

Contribution of the Authors

Thiago Donizete Da Silva-José participated in the study design, execution, data analysis, statistical analysis, manuscript drafting, and critical discussion. Karina Janett Juárez-Rendón participated in the study design, manuscript drafting, and critical discussion. Jesús Alejandro Juárez-Osuna and Angela Porras-Dorantes participated in the acquisition of data and study execution. Adán Valladares-Salgado, Miguel Cruz, Miriam Gonzalez-Ibarra, and Ana G. Soto participated in the acquisition of data and samples. María Teresa Magaña-Torres and Lucila Sandoval-Ramírez participated in the study design, data analysis, and critical discussion. José Elías García-Ortiz participated in the study

Table 2 *p*-values of the comparison from allelic frequencies of the dup-24 bp polymorphism between pairs of populations analyzed*

Population	Purepecha	Tarahumara	Huichol	Mayan	Tenek	Nahua	Mexican mestizo	Asiatic	European	African	Peruvian mestizos	Indigenous Amerindian	Indigenous Amazonian
Tarahumara	0.77178												
Huichol	0.75821	0.61851											
Mayan	0.42037	0.21000	0.76880										
Tenek	0.74951	0.95429	0.82458	0.41009									
Nauha	0.28584	0.23058	0.61454	0.65843	0.47287								
Mestizo ⁹	0.08031	0.20447	0.01816	0.00041	0.12841	0.00108							
Asiatic ¹⁰	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000						
European ¹⁰	0.00210	0.00258	0.00003	0.00000	0.00000	0.00000	0.05298	0.00000					
African ¹⁰	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000				
Mestizos	0.05939	0.00294	0.04577	0.10112	0.00901	0.00774	0.00000	0.07108	0.00000	0.00000			
Peruvian ¹²													
Indigenous Amerindian ¹²	0.72112	0.61021	0.25594	0.06524	0.43566	0.05416	0.19040	0.00000	0.02372	0.00000	0.00348		
Amazonian indigenous ¹²	0.04782	0.00251	0.02309	0.04107	0.00509	0.00334	0.0000	0.38909	0.00000	0.00000	0.84083	0.00334	
Quechua indigenous ¹²	0.30761	0.19435	0.54261	0.72166	0.31844	0.64632	0.02705	0.05346	0.00000	0.00000	0.59694	0.08153	0.36634

**p* < 0.05

design, execution, data analysis, statistical analysis, manuscript drafting, and critical discussion.

Informed Consent

All procedures were in accordance with ethical standards of the responsible committee on national human experimentation and with the Helsinki Declaration of 1975, as revised on 2000 and 2013. Written informed consent was obtained from all individuals for being included into the study.

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