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Association study from Tight Junction Protein-1 gene SNV rs2291166 with the metabolic parameters from Fitness in body building from Oaxaca, México

[1] Héctor Aguilar-Aguilar, [2] Elva Montero-Toledo, [3] Luis Abel Solano Santiago, [4] Adolfo Demetrio Gómez-Hernández, [5] Luis Abel Solano-Ramirez, [6] Luis Fernando Martínez-Miguel, [7] Emanuel de Jesús Cruz-Cortes, [8] Luis Alberto Hernández-Osorio, [9] Sergio Alberto Ramirez-Garcia

[1, 3, 4, 5, 6, 7] Facultad de Cultura Física y Deporte de la Universidad Autónoma Benito Juárez de Oaxaca, México
[2, 8, 9] Facultad de Ciencias Químicas de la Universidad Autónoma Benito Juárez de Oaxaca, México

Corresponding Author: **Sergio Alberto Ramirez-Garcia**

Abstract

Background: Considering that the *TJP1* (tight junction protein type 1 gene) allele variant translate 1334-aminoacid change asp [G*A*C] >ala [G*C*C], SNV *rs*2291166 of which has already been associated with albuminuria and other biochemical markers of cardiovascular risk, such as HOMA index. Aim. The main objective was to investigate the relationships of the genotypes of the genetic variant SNV rs2291166 of *TJP1* gene with metabolic and biochemical fitness parameters in in body building subjects. Method and subjects. 1014 Adult in body building patients were included in the study. Single nucleotide variation (SNV) genotyping was performed by chain reaction of the allele-specific polymerase. Biochemical parameters were performed using by direct photometry. Results. The glycosylated hemoglobin A1C levels were lower in homozygous G carriers. The carrier's heterozygote, have increased systolic tension levels and body mass index. Conclusion. The SNV rs2291166 from *TJP1* gene is a genetic marker that influences the factors of cardiovascular risk in body building.

Keywords: A1c-glycosylated Hemoglobin, Cardiovascular Risk, Obesity, Overweight, Paracellular Transport, Tight Junctions

Introduction

Cells in the biological tissues are connected between them through an intercellular union system, which is classified into three different types: tight, gap and adherent junctions. Tight junctions are composed of transmembrane, cytoskeletal, and cytoplasmic plaque proteins, they perform substantive physiological functions of protecting adjacent cells, against fluids and extracellular material, and paracellular transport [1-3].

In humans *TJP1* gene encodes two transcript variants of tight junction protein 1 (also known as ZO-1), which belongs to the family of zonula occludens proteins. (ZO-1, ZO-2, and ZO-3) ZOs is a cytoplasmic plaque protein that is directly involved in paracellular transport, because contains a PDZ domain which forms a binding site for other tight junction proteins [3]. The *TJP1* allele variant translate a 1334-aminoacid change [asp [G*A*C] >ala [G*C*C], SNV *rs*2291166; chr15:29716773 (GRCh38.p12)] of which has already been associated with albuminuria, and biochemical markers of cardiovascular risk (CV) [4-6]. Besides that, it has been identified that ZO-1 is in two modular isoforms which allows them to act to limit movement of substances through the paracellular space, characteristic that favors the transport of nutrients (such as glucose), functioning as scaffolds at the intestinal epithelium [4-6].

In the scenario of intestinal cells, the triglycerides, are hydrolyzed by the lipoprotein lipase enzyme, and its products increase the permeability of ZO- [4], it is based on previous reports that rats exposed to a high-fat diet show both, a significant increase in the formation of atheroma with lower expression of Cx43 (Conexin-43) and ZO-1, and higher penetration of CD14 monocytes in the heart and aorta; it is worth noting that the formation of atheroma could be due to the loss of TJP's [5].

On the other hand, it has been shown that ZO-1 regulates the tension on the adherent junctions based on VE-cadherins, cell-cell stress, cell migration, and angiogenesis *in vitro* and *in vivo* [2], suggesting that the two functional variants of *TJP1* gene could influence the levels of biochemical parameters, such as glucose, A1c-glycosylated hemoglobin (HbA1c), serum insulin, cholesterol and triglycerides, as well as other polymorphism in gene encoding the intestinal transporter type 2 of the fatty acids protein, *FABP2*, whose T54 allele is associated with the before cited metabolic markers in obese Mexican subjects [7].

Previously, we reported (*in silico* study), that the 1334-aminoacid change [asp [G*A*C] >ala [G*C*C] protein favors a structural putative change, generating two iso-forms of which the ancestral isoform p.1334D presents greater conformational stability [8]. The ancestral allele (G) was found high in Amerindian Mexican Zapoteca, Chinanteca and Mestizos [8-10]. Preliminary report in Chinanteca population, the heterozygote (T/G) genotype for this genetic variant is related to the HOMA index [10].

Since the tight junction proteins are concurrently correlated with the fitness metabolic function in body building, in this study we proposed that the allele distribution of SNV rs2291166 within the *TJP1* gene could be associated with the levels from the metabolic markers from fitnees in adults than practice body building from Oaxaca, México.

Material and Methods

Study design and subjects**.** The present research is a descriptive cross-sectional study. The sample population was 1014 adults' males body building usuries come to our center every year for their check-up from the Center from Research Traslacional and Medical Precision from the Sierra Sur Oaxaca, México (age range 21-55 years old), enrolled in the period january 2012-march 2022. The subjects were natives from Zapotecan ancestry. All subjects were enrolled in the original research project entitled. The study was approved by local research and bioethics committees. This study was conducted in accordance to the updated Helsinki declaration. Consent informed was obtained from all of the participants.

Clinical and biochemical evaluation

Anthropometric markers. Blood pressure was measured three times with a fixed mercury baumanometer, with the subject in the sitting position for 15 minutes before the evaluation. Waist circumference (WC), height, and weight measurements were recorded. The body mass index (BMI) was calculated by the Quetelet index; kg/m^2= weight (kg)/height^2(m).

Biochemical markers. Individuals included in the study were fasting for 12 h before collecting the peripheral blood samples and then centrifuged at 1509 RCF (Rotanta 460R; Andreas Hettich GmbH & Co. KG., Germany) for 10 min at 20°C. Serum was collected and stored at −20°C. Glucose concentration and lipid profile (mg/dL) including triglycerides (TG), total cholesterol (TC), HDLc, low-density lipoprotein cholesterol (LDLc) were measured by direct photometry. The serum insulin was determined using commercial enzyme-linked immunosorbent assays (ALPCO, USA). The glycosylated hemoglobin A1C percentage was obtained by immunochromatography. The homeostasis model assessment-insulin resistance (HOMA-IR) = [fasting serum glucose mg/dL × (fasting serum insulin µUI/mL)/405], cut point of 2; quantitative insulin sensitivity check index, QUICKI = [1/(log(fasting insulin, IU/mL) + log (fasting glucose, mg/dL)], HOMA-B = 20 × insulin (µUI/mL)/[glucose (mmol/L) – 3.5] and HOMA-B (HOMA-Beta) [11-12]. The CV risk was measured according to the Framingham III study [13].

Genotyping

Genomic DNA was isolated from 5 mL of peripheral blood into a tube with ethylene diamine tetra acetic acid (EDTA), which was collected from each teen individual. In accordance to the manufacturer's protocol, a commercial kit (Gene Catcher, Invitrogen) was used to isolate the DNA. DNA integrity was verified by agarose electrophoresis at 0.8% and DNA concentration was evaluated in a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Genotyping of variant (formerly polymorphism) rs2291166 of the *TJP1* gene was performed by the specific allele polymerase chain reaction (PCR-PASA), as previously validated [10]; the primers forward for PCR amplification were designed in the minus strand, *locus* chr15:29716773 (Genosys Sigma-Aldrich), primer reverse in the plus strand:

Forward: W1G5´-CTTCATCTTCTTCAGGTT-3´
Forward: FW2A5´-ATATTCTTCATCTTCTTCAGGTG-3´
Reverse: W35´-GTCATTCATTATCTGTTAGG-3´

DNA amplification was carried out with five minute (initial denaturing), followed by 30 cycles: at 95˚C, for 30s, 48°C, 45s, at 72°C, 30s, with a final extension of 72°C, 5 min (Termocycler Termociclador MultiGene™ OptiMax, 120V Labnet™). The reaction mixture; KCl buffer 2.5 µl (1X), MgCl 21.5 µl (25 mM), 0.56 µl of dNTP's (0.2 mM), 0.5 µl of each primer (25 pmoles), 2 µl of DNA template (100 ng), DNA Pol Taq 0.3 µl (3 U/ul) (SBS Genetech CO.,Ltd), and 17.20 µl of water molecular biology grade, final reaction 25 µl. The PCR products were separated by electrophoresis in Acrylamide/N-metylbisacryilamide gel (14cm by 20cm) at 19:1 ratio, 12%, running with buffer TAE 1.0X for 11.5 hours at 200V, 80-84 Ma [10]. The gels were stained with a solution contained silver nitrate 0.11 gr, ethanol 100ml, acetic acid 250µl and the products were distinguished by sizes; that of 102 pb corresponds to the T allele, whilst that of 107 pb corresponds to the G allele, as previously reported [10].

Statistical analysis

For each of the parameters of the metabolic syndrome (biochemical and anthropometric), the mean and standard deviation was calculated by genotypes. The differences between the genotype groups were compared by the T student's test, ANOVA and post-hoc Dunnet test.

Results

The ancestral allele (G) had a frequency of 11%, while the wild type allele (T) had a frequency of 89% of the analyzed population. On addition, the ancestral genotype (GG) had a frequency of 2%, the heterozygote, 18% and the wild homozygote (TT), 80% (Table 1 and 2).

When stratifying the population by genotypes, SBP (mmHg) levels are increased significantly in the carrier's heterozygote versus homozygote (Table 1), as well as BMI. Glycosylated hemoglobin A1c levels were lower in homozygous G carriers as well as in the TAS and BMI levels.

Table 1: Metabolic parameters according to SNV rs2291166 of the *TJP1* gene

	GG(n=18)		**GT** (n=180)		**TT** (n=816)		**P value**
Parameters	**Average**	**De.Est**	**Average**	**De.Est**	**Average**	**De.Est**	
Age	14	2.82	16.6	0.98	14.82	2.57	0.0305
Weight	73.60	12.58	77.66	12.21	69.28	12.58	0.0666
Height	1.55	0.04	1.6	0.06	1.54	0.10	0.1087
BMI (kg/m2)	14.01	1.979	30.31	3.33	28.54	4.63	0.0002
Waist circumference (cm)	94.73	25.45	89.48	8.99	88.21	8.03	0.5868
TAS (mmHg)	95.00	7.07	112.33	15.68	102.11	13.86	0.0315
TAD(mmHg)	60.00	10.10	64.00	9.10	61.02	10.67	0.5916
Insulin(mU/mL)	12.71	5.30	21.34	7.29	22.32	7.27	0.3929
Glucose(mg/dL)	94.36	22.62	98.93	15.34	94.69	16.88	0.6694
HOMA-IR	3.47	0.50	5.16	1.85	5.25	2.07	0.6633
HOMA-BCF	97.32	30.22	232.77	122.12	328.66	269.10	0.2874
Total cholesterol (mg/dL)	106.51	6.36	133.42	24.22	131.97	26.16	0.3739
Cholesterol-HDL(mg/dL)	29.59	4.17	36.20	18.33	32.29	10.33	0.4894
Cholesterol-LDL (mg/dL)	79.23	22.62	73.00	12.75	75.32	20.16	0.8737
Triglycerides (mg/dL)	123.50	4.94	129.8	78.79	121.60	56.76	0.8943
RCV	4.45	0.49	4.29	1.57	4.52	3.82	0.9743
HbA1c	4.65	0.21	5.22	0.34	5.40	0.40	0.0145

Abbreviations: De.Est, standard deviation; Cardiovascular risk, RCV; Homeostasis model assessment insulin resistance, HOMA-IR; percentage of beta cell, HOMA-BFC;HbA1c, glycosylated hemoglobin A1c

Concerning the body weight factor, the carrier's homozygotes T had the lowest body weight, followed by the carrier's homozygotes G (Table 1). There is a lack of associations to the other parameters or cardiovascular risk with the genotype.

Discussion

This is the first study that suggests that the variant rs2291166 of the *TJP1* gene influences glycosylated hemoglobin levels in the subjects than practice body building, mainly is associated with metabolics fitnees parameters such as overweight or obese. Considering that obesity is stronger related to type 2 diabetes mellitus, the variant polymorphism rs2291166 could be a predictor of diabetes mellitus type 2, further research is necessary to explain this relationship, or a follow-up study and association to diabetes. At the moment it could be related to the greater para-cellular passage of glucose in the small intestine, since the studied variant leads to a change on the structure of ZO-1 due to amino acid change D>A, related to this phenotype [3, 8]. This pathogenic effect has to be demonstrated *in vitro*, but was beyond the scope of this study.

The relationship between the *TJP1* with obesity may be due to morphogenesis of the nucleus of the satiety at the central nervous system as wells as adipocyte [10]. In addition, the association to systolic blood pressure levels, can be explained by the essential role of TJPs in angiogenesis and permeability of the endothelial vascular barrier as well as adipogenesis [1-2]. Because of this role of TJPs, more research is need on the variants of *TJP1* gene in other populations who have different genetic background and feeding.

This polymorphism had analyzed in people with Zapoteca ancestry. We found a high frequency of the allele of ancestral genotype (G), which is common in previously studies among both Amerindian groups in Oaxaca México (Zapoteca or Chinanteca population) and African ethnic groups [8-10]. The present work includes an important number of probands suggesting a funder effect between Amerindian Mexican and African population by ancestral allele [8-10]. In this study, of more than 2000 chromosomes analyzed, 180 favored G carriers, a genotype infrequently reported in the SNP bank (populations descendants of the Clinseq project Europeans n=1323) and frequently in African 1000 Genomes super population n=1322), with a fewer number of people see Table 2 [8-10].

Table 2: World frequency of the SNV rs2291166 of the *TJP1* gene

		Gentotype detail counts			**Alleles counts**	
Individual Group Population	**Chrom. Sample Cnt**	**TT**	**TG**	**GG**	**T**	**G**
Latin American ethnic groups						
Mexican Ancestry from Los Angeles USA	100	0.94	0.06	0	0.97	0.03
Coriell Cell Repository (CCR), Hispanic	44	1	0	0	1	0
Mexican mestizos from Northern and Western from México	946	0.957	0.041	0.002	0.978	0.022
Mexican ancestry Zopoteca from Oaxaca, México	318	0.9812	0.0188	0	0.9906	0.0094
***This study in Oaxaca, México.**	2028	0.804	0.178	0.018	0.8935	0.1065
Ethnic groups in the United States of North America						
Chinese descent from the Coriell Cell Repository were selected from the Han People of Los Angeles Panel	48	0.958	0.042	0	0.979	0.021
Coriell Cell Repository are primarily of European American descent	48	0.792	0.208	0	0.104	0.896
Utah residents with Northern and Western European ancestry from the CEPH collection	120	0.867	0.133	0	0.933	0.067
Utah residents with Northern and Western European ancestry from the CEPH collection	226	0.885	0.115	0	0.942	0.058

African ancestry in Southwest USA	98	0.98	0.02	0	0.99	0.01
African American descent from the Coriell Cell Repository	46	0.913	0.087	0	0.957	0.043
African american, Coriell Cell Repository	30	0.8667	0.1333	0	0.967	0.033
Gujarati Indians in Houston, Texas	176	0.864	0.136	0	0.932	0.068
European populations						
European descent from the ClinSeq project	1323	0.894	0.101	0.005	0.945	0.056
Toscans in Italy.	176	0.943	0.057	0	0.972	0.028
European Coriell Cell Repository	30	0.867	0.133	0	0.967	0.033
African populations or ethnic groups						
Luhya in Webuye, Kenya.	180	0.978	0.022	0	0.989	0.11
Maasai in Kinyawa, Kenya	286	0.895	0.084	0.021	0.937	0.063
Yoruba in Ibadan, Nigeria	224	0.982	0.018	0	0.991	0.009
Yoruba in Ibadan, Nigeria, Hap Map	120	0.983	0.017	0	0.992	0.008
Asian populations or ethnic groups						
Unrelated Han Chinese in Beijing, China	86	0.977	0.023	0	0.988	0.012
Han Chinese in Beijing, China, HapMap	80	0.95	0.05	0	0.975	0.025
Asiáticos de la tribu Han de Beijing, China Hap-Map JPT	90	0.978	0.022	0	0.989	0.011
Asiáticos de Tokio Japón, Hap Map	172	0.965	0.035	0	0.983	0.017
Asiáticos de Tokio Japón	90	0.956	0.044	0	0.978	0.022
Chinos en Metropolitan Denver, Colorado	168	0.917	0.083	0	0.958	0.042
DNA Asian was extracted from complete hydatidiform mole	74	0.9594	0.0405	0	0.95945	0.0405
Other Populations or ethnic groups						
Coriell Apparently Healthy	68	0.73252	0.2352	0.0294	0.8529	0.147

Note: Edited from SNP Bank, last access 01-07-2022. Abbreviations: ND=Data not available

Lack of associations to the other parameters from the fitness or cardiovascular risk with the genotype may be related to other environmental factors such as diet, in this sense, a correlation was recently found between the SNV of *TJP1* that we analyze here, with the FAO dietary quality index in the metabolic risk parameters or pathogenic mechanism of the atherosclerosis [5, 10]. But also, by other genetic variants that were not analyzed study from *TJP1* gene and other atherosclerosis genes related, these will be studies of later in other research Works.

Recently in was reported *TJP1* Mutations in arhythmogenic Cardiomyopathy, p.Y669C, ZO-1-p.R265W p.S329L, p.D360V [14]. This can be explained because the *TJP1* protein (ZO-1), is a scaffolding protein that localizes to the intercalated disc of cardiomyocytes and has been interact with related proteins, as the connexin43, N-cadherin, αT-catenin, and actin, also presents in the vascular cytoskeleton [14]. These rare variants in human are a frontier in the cardiometabolic risk and atherosclerosis in different practice sports.

In conclusion the SNV rs2291166 from *TJP1* is a genetic marker that influences blood pressure and hemoglobin glycosylated A1C levels in in body building from Oaxaca, México. Considering that it was analyzed in a significant population group, it is suggested to analyze its effect on cardiovascular risk parameters in other sport practice, since it could be considered a marker of prediabetes by influencing the levels of glycated hemoglobin a1c. The cardiovascular risk and its spectrum of alterations in different sports is very important to estimate and detect the associated complications, because it can be a cause of sudden early death, or deteriorate sports performance, hence the importance of our study that presents a marker. genetic that allows the early detection of cardiac problems, which are a very common clinical problem in athletes

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Conflict of Interest. The authors declare that they have no conflict of interest.

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