Received: 26 June 2019 | Revised: 30 March 2020 | Accepted: 31 March 2020

DOI: 10.1111/ijpo.12641

ORIGINAL RESEARCH

Pediatric OBESITY WILEY

Association of *AMY1A/AMY2A* copy numbers and AMY1/AMY2 serum enzymatic activity with obesity in Mexican children

Miguel Vázquez-Moreno[1,2] | Aurora Mejía-Benítez[2] | Tanmay Sharma[2] | Jesús Peralta-Romero[1] | Daniel Locia-Morales[1] | Miguel Klünder-Klünder[3] | National Obesity Network Mexico[4] | Miguel Cruz[1] | David Meyre[2,5]

[1]Hospital de Especialidades, Centro Médico Nacional Siglo XXI del Instituto Mexicano del Seguro Social, Unidad de Investigación Médica en Bioquímica, Mexico City, Mexico

[2]Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, Canada

[3]Departamento de Investigación en Salud Comunitaria, Hospital Infantil de México Federico Gómez, Mexico City, Mexico

[4]Instituto Mexicano del seguro social, Mexico City, Mexico

[5]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Canada

Correspondence
David Meyre, Department of Health Research Methods, Evidence, and Impact, McMaster University, Michael DeGroote Centre for Learning & Discovery, Room 3205, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.
Email: meyred@mcmaster.ca

Miguel Cruz, Hospital de Especialidades Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Unidad de Investigación Médica en Bioquímica, Av. Cuauhtémoc, 330 C.P. 06725, México City. Mexico.
Email: mcruzl@yahoo.com

Funding information
Canada Research Chairs; Consejo Nacional de Ciencia y Tecnología; Instituto Mexicano del Seguro Social; Ciencias Médicas Odontológicas y de la Salud PhD program from Universidad Nacional Autónoma de México; Mexican Institute of Social Security under the program of Priority Health Topics 2017

SUMMARY

Background: Mexican children are characterized by a high-starch intake diet and high prevalence of obesity.

Objectives: To investigate the association of *AMY1A/AMY2A* copy numbers (CNs) and AMY1/AMY2 serum enzymatic activity with childhood obesity in up to 427 and 337 Mexican cases and controls.

Methods: Anthropometric and dietary starch intake data were collected. CN of *AMY1A/AMY2A* and AMY1/AMY2 serum enzymatic activity were determined using droplet digital PCR (ddPCR) and enzymatic colorimetry, respectively. An individual participant level data meta-analysis of association between *AMY1A* CNVs and obesity was also performed.

Results: A positive association between *AMY1A/AMY2A* CNs and their corresponding AMY1/AMY2 serum enzyme activity was observed in children with normal weight and obesity. The serum enzyme activity of AMY1 and AMY2 was negatively associated with childhood obesity risk, and the association was restricted to kids eating medium/high amount of starch ($P_{interaction}$ = .004). While no association between *AMY1A* and *AMY2A* CNs and childhood obesity was observed in our sample, we confirmed a significant association between *AMY1A* CN and obesity in a meta-analysis of 3100 Mexican children.

Conclusions: Our data suggest that genetically determined salivary and pancreatic amylase activity can increase/decrease the risk of obesity in Mexican children, this effect being blunted by a low-starch diet.

KEYWORDS

amylase, childhood obesity, copy-number variants, dietary starch intake, enzyme activity, Mexican population

Investigators listed in Appendix.

 © 2020 World Obesity Federation

1 | INTRODUCTION

The global prevalence of obesity has nearly tripled since 1975 according to the world health organization, but it affects certain countries more than others. Mexico has the second highest rate of obesity in the world, after the United States. In 2016, the national survey of health and nutrition of Mexico reported obesity prevalence rates of 15.3%, 13.9%, and 33.3% among children between the ages of 5 and 11 years, 12 and 19 years, and adults, respectively.[1] Childhood and adolescent obesity are associated with early-onset of co-morbidities (eg, type 2 diabetes, cardiovascular disease, cancer), and premature all-cause adult mortality.[2] While some therapeutic interventions are available for children (eg, lifestyle and behavioral modifications), there has been limited success in curbing the obesity epidemic so far.[3] Understanding the causes of obesity, especially in high-risk pediatric populations, has the potential to improve prediction, prevention and treatment of the disease.[4]

The recent obesity epidemic in Mexico can be explained in part by a rapid nutritional transition to Western diet and a decrease in average physical activity induced by the fast urbanization of the country.[5,6] However, not everyone exposed to an "obesogenic environment" becomes obese, and large inter-individual differences in body mass index (BMI) are observed among the Mexican population.[1,7] These differences can be attributed to biological factors: in utero programming, sex, age, ancestral background, gut microbiome, epigenetic and genetics.[4]

Twin and family studies in diverse ethnic groups, including Latino Americans, suggest that 40% to 75% of BMI variation is driven by genetic factors.[8] While common single-nucleotide variants account for 30% to 40% of heritability for BMI in children and adults,[9,10] structural variations may explain part of its missing heritability.[11] Rare and frequent copy number variants (CNVs) have been associated with childhood and adult obesity, predominantly in populations of European ancestry.[4] However, most of these associations have been challenging to reproduce.[12]

This is illustrated by the copy numbers (CNs) of the human salivary (*AMY1A*) and pancreatic (*AMY2A*) amylase genes.[13,14] In 2014, Falchi et al evidenced an association between low CN of the *AMY1A* gene, low serum amylase enzyme levels, and high risk of adult obesity in European and East Asian populations.[13] However, the accuracy of the q-PCR CN measurement method used in the Falchi et al study has been questioned,[14-17] and alternative methods (eg, droplet digital PCR [ddPCR] in combination with or without the classic q-PCR, PCR junction fragment assay) have been proposed to genotype the CN at the *AMY1A* and *AMY2A* genes.[14-17] In European populations, there is a fundamental structural distinction between haplotypes containing odd or even numbers of *AMY1* gene units, which is in turn coupled with CN in pancreatic amylase genes *AMY2A* and *AMY2B*.[16] The association between CN at the *AMY1A* gene, genotyped using ddPCR, and BMI/obesity has been replicated in some but not all adult European and East Asian cohorts.[14,15,18] With respect to childhood obesity, associations between *AMY1A* CN and childhood obesity status have been described in European and African-American populations.[17,19-21] Additionally, an association between serum AMY1 and AMY2 enzymatic activity and BMI has also been observed in adult Europeans.[17]

Studying the associations of CNs at the *AMY1A* and *AMY2A* genes and serum AMY1 and AMY2 enzymatic activity with obesity-related traits in the Mexican population is of great interest, considering their high consumption of dietary starch.[22,23] This is owing to the fact that natural selection has resulted in a higher number of *AMY1A* copies in populations exposed to high-starch consumption,[24] and dietary starch intake has been shown to potentially modify the relation between *AMY1A* CN and BMI.[25] Mejia-Benitez et al reported an association between low *AMY1A* CN, measured by digital qPCR, and obesity in Mexican children.[26] More recently, *AMY1A* CN, measured by ddPCR, was associated with childhood and adult obesity in an independent Mexican sample.[27] While promising, these associations need further confirmation at this stage. In addition, the association of CN at the *AMY2A* gene and AMY1/AMY2 serum enzymatic activity with obesity has never been investigated to date in the Mexican population. Hence, this study aimed to assess: (a) the correlation between serum enzymatic activity of AMY1 and AMY2; (b) the association between serum enzymatic activity of AMY1 and AMY2 and childhood obesity; (c) the correlation between CNs of *AMY1A* and *AMY2A* genes; (d) the association between CNs of *AMY1A* and *AMY2A* genes and serum enzymatic activity of salivary and pancreatic amylase; (e) the association of CNs of *AMY1A* and *AMY2A* genes with childhood obesity; (f) the interaction between CNs of *AMY1A* and *AMY2A* genes, enzymatic activity of salivary and pancreatic amylase, starch intake and obesity risk; and (g) to perform a meta-analysis of association of the CN of *AMY1A* gene with childhood obesity in the Mexican population (Figure 1).

2 | METHODS

2.1 | Study participants

The research was approved by the ethics committee of the Mexican National Health Service ("Instituto Mexicano del Seguro Social" CONBIOETICA-09-CEI-009-20160601) and was conducted in compliance with the Declaration of Helsinki. Child assent was obtained and parents (or legal guardians) provided written informed consent prior to enrollment into the study. In our discovery sample, a total of 764 children (427 with normal-weight and 337 with obesity) between the ages of 6 and 12 were enrolled from three different States in Mexico (Campeche, Oaxaca, and Mexico City) to evaluate the association of serum enzymatic activity of AMY1 and AMY2 ($N_{normal\text{-}weight}$ = 427; $N_{obesity}$ = 337) and CN of *AMY1A* and *AMY2A* ($N_{normal\text{-}weight}$ = 277; $N_{obesity}$ = 174) with obesity. The study was conducted from 2011 to October 2018 (Table 1).

In our replication sample, as part of the National Obesity Network Mexico initiative, we also collected genetic (*AMY1A* CN) and clinical data for 1131 children (627 with normal-weight and 504 with obesity), between the ages of 6 and 12, that were enrolled from 14 different States in Mexico (Baja California Sur, Campeche, Estado de Mexico, Guanajuato, Hidalgo, Michoacán, Nayarit, Nuevo León, Oaxaca, Puebla, Queretaro, Sinaloa, Sonora and Tamaulipas). The recruitment was conducted between June 2016 and October 2018 (Table S1).

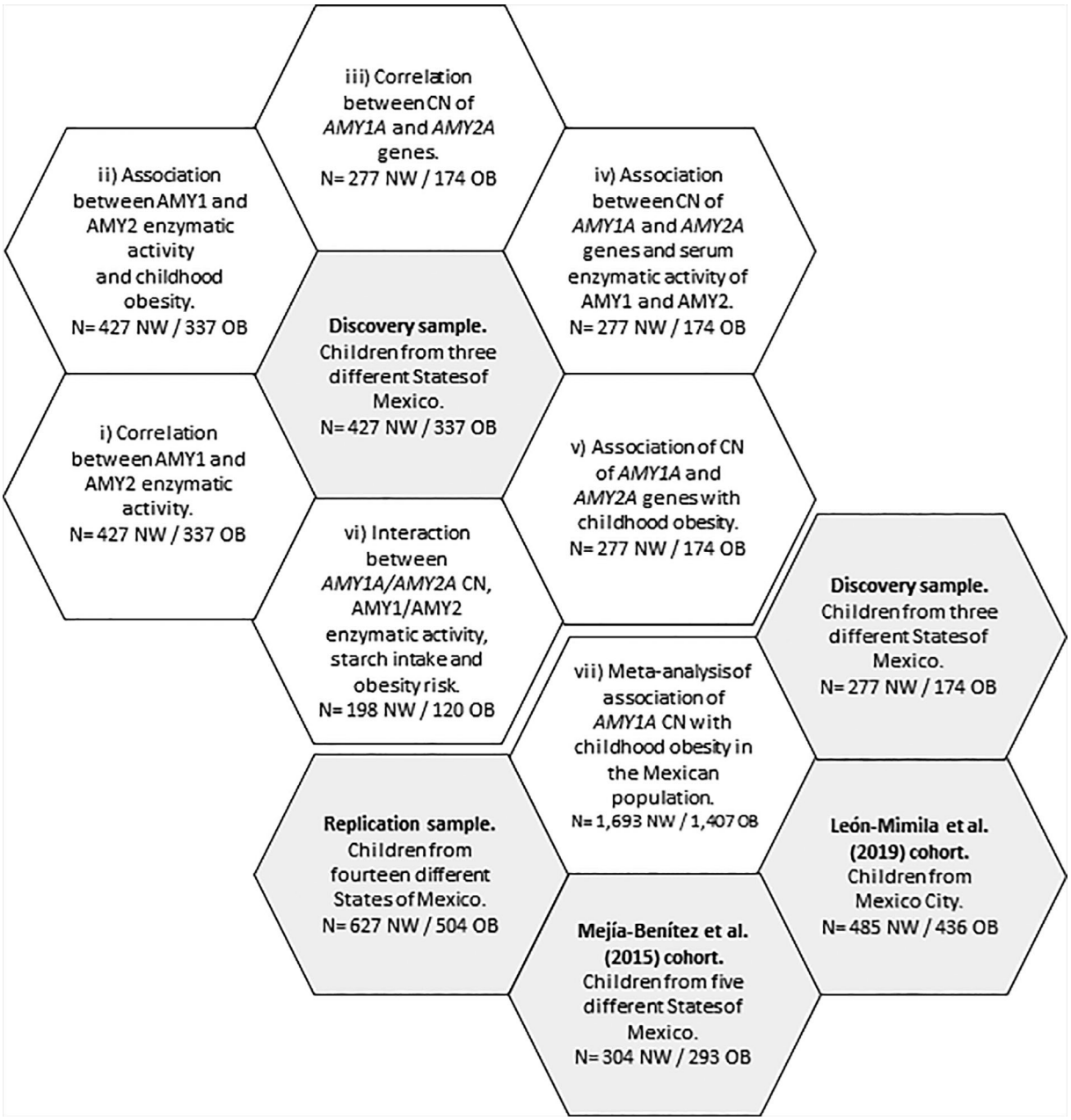

FIGURE 1 Flowchart of the study design. The study aims are represented in a white background andthe sample(s)/cohort(s) are represented in a grey background. Abbreviations: AMY1, salivary amylase; AMY2, pancreatic amylase; CN, copy number; NW, normal weight; OB, obesity

2.2 | Anthropometric measurements

Subjects were instructed to wear light clothing and remove their shoes for the height and weight measurements. All measurements were performed by a trained nurse. Weight was measured using a digital weighing scale (Seca, Hamburg, Germany) to the nearest 0.1 kg and height was measured using a Seca 225 stadiometer to the nearest 0.1 cm. BMI was calculated as weight (kg)/height $(m)^2$ with percentiles for age and sex derived from the Centers for Disease Control and Prevention (CDC) 2000 references.[28] Children with a BMI ≥5th and < 85th percentile were classified as normal weight, those with a BMI ≥85th and <95th percentile as overweight, and those with a BMI ≥95th percentile as obese. We only enrolled children with normal-weight and obesity in this study (case control study design).

2.3 | Processing of blood samples

A blood sample for DNA extraction was collected in participants of the discovery and replication studies, following a 12-hour fast. In Campeche, Mexico City and Oaxaca states, two blood samples were taken, one of 4 mL to extract genomic DNA and one of 5 mL to

TABLE 1 General characteristics of Mexican children with normal weight and obesity included in the discovery sample

Analysis/Trait	Normal weight	Obese	*P* value
AMY1/AMY2 enzymatic activity analysis (discovery sample)	**N = 427**	**N = 337**	**NA**
Female, N (%)	250 (58.5%)	153 (45.4%)	**0.043**
Age (years)	9.0 ± 1.9	9.2 ± 1.8	0.938
BMI (kg/m^2)	16.9 ± 3.0	24.4 ± 2.7	**5.3×10^{-32}**
SDS-BMI	0.24 ± 0.65	2.03 ± 0.77	**2.1×10^{-43}**
AMY1 (IU/L)	40.7 ± 20.5	35.6 ± 19.4	**0.001**
AMY2 (IU/L)	22.0 ± 7.0	20.0 ± 6.4	**5.7×10^{-5}**
***AMY1A/AMY2A* copy number analysis (subset of the discovery sample)**	**N = 277**	**N = 174**	**NA**
Female, N (%)	145 (52.3)	77 (44.3)	0.094
Age (years)	9.7 ± 1.9	9.5 ± 1.8	0.325
BMI (kg/m^2)	17.1 ± 3.1	24.4 ± 2.9	**1.9×10^{-31}**
SDS-BMI	0.23 ± 0.62	2.01 ± 0.66	**6.2×10^{-43}**
AMY1 (IU/L)	36.8 ± 19.5	31.6 ± 19.6	**0.007**
AMY2 (IU/L)	21.7 ± 7.3	19.5 ± 6.9	**0.002**
AMY1A CN	6.8 ± 2.6	6.7 ± 2.5	0.510
AMY2A CN	1.9 ± 0.4	1.9 ± 0.4	0.434
Starch intake interaction analysis (subset of the discovery sample)	**N = 198**	**N = 120**	**NA**
Female, N (%)	101 (51.0)	57 (47.5)	0.515
Age (years)	9.8 ± 2.0	9.7 ± 2.0	0.540
BMI (kg/m^2)	16.7 ± 3.2	25.0 ± 2.5	**1.8×10^{-34}**
SDS-BMI	0.11 ± 0.65	2.09 ± 0.71	**2.4×10^{-40}**
AMY1 (IU/L)	36.3 ± 20.5	29.7 ± 19.4	**0.012**
AMY2 (IU/L)	21.1 ± 7.0	18.2 ± 6.4	**0.001**
AMY1A CN	6.6 ± 2.5	6.5 ± 2.6	0.443
AMY2A CN	1.95 ± 0.46	1.91 ± 0.44	0.681
Energy intake (MJ/d)	9.4 ± 3.4	9.0 ± 3.3	0.576
Starch (g/d)	92.6 ± 35.7	93.6 ± 37.4	0.815

Abbreviations: BMI, body mass index; SDS-BMI, -age and -sex adjusted SD scores of BMI; AMY1, salivary amylase; AMY2, pancreatic amylase; NA, not analyzed.

Notes: Data are expressed as mean ± SD or N (%). Difference in sex ratios was analyzed using the $\chi 2$ test. Differences in means tests were analyzed using Student's *t*-tests. Significant *P* values ($P < .05$) are reported in bold.

extract the serum and to analyze the enzymatic activity of salivary and pancreatic amylase. Blood sample tubes for enzymatic activity analyses were kept in a vertical position for 30 minutes and centrifuged at 15 000 RPM for 10 minutes in the clinical laboratory from each local recruitment center. The serum was extracted and stored in 500 μL aliquots together with the blood sample for DNA extraction at −20°C. The serum aliquots and blood sample tubes for DNA extraction were sent to the laboratory of the Mexican Institute of Social Security in Mexico City by project collaborators in an airtight cooler with at least 10 kg of dry ice. DNA extraction and enzymatic activity analyses were centralized in laboratories from Mexico City.

2.4 | Measurement of AMY1/AMY2 serum enzymatic activity

The participants were scheduled for clinical laboratory evaluation following a 12 hours overnight fasting period. Serum enzymatic activities of total amylase and AMY2 were measured by enzymatic colorimetric assay, with a COBAS Icobas 8000 modular analyzer series (Hoffman-La Roche. Basel, Switzerland). Experiments were performed in the CENAREM core laboratory facility (Mexico City). The enzymatic activity of AMY1 was calculated by subtracting the activity of AMY2 from the activity of total amylase. Only children with enzymatic activities of

13 to 53 U/L of AMY2 and 29 to 99 U/L of total amylase were included for analysis, based on previous recommendations.[29]

2.5 | DNA extraction and *AMY1A* and *AMY2A* CN detection

Genomic DNA of peripheral mononuclear cells was obtained from children. DNA was purified using the AutoGenFlex STAR (Auto-Gen, Holliston, MA, USA), and the purity and integrity were verified by 260/280 nm measurements (BioTek Instruments, Winooski, VT) as well as by electrophoresis in 0.8% agarose gels stained with ethidium bromide. The CN detection was performed by ddPCR using the QX200 system (Bio-Rad Laboratories, Hercules, CA) following the protocol proposed by Bonnefond et al.[17] Each duplex reaction of 40 μL contained 0.5 of U HindIII (Thermo Fisher Scientific, Waltham, MA), 11 μL of ddPCR SuperMix for Probes no dUTP (Bio-Rad), 24 ng of DNA, 1.1 μL of TaqMan assay targeting *AMY1A* or *AMY2A* (Hs07226362_cn or Hs04204136_cn, respectively; Thermo Fisher Scientific) and 1.1 μL of TaqMan assay targeting the reference RNase P assay (Human RNase P #4403328; Thermo Fisher Scientific). Genomic DNA was digested for 5 minutes at 20°C before submitting them to the Droplet Generator Oil (Bio-Rad) in the QX200 Droplet Generator (Bio-Rad) and performing the PCR amplification in 96-well reaction plate (Bio-Rad) with a Thermal Cycler (Bio-Rad). Results from PCR amplification were read using a QX200 Droplet Reader (Bio-Rad) and analyzed with the QuantaSoft Analysis Pro software (version 1.0.596, Bio-Rad). DNA extraction and CN genotyping were performed at the IMSS laboratory in Mexico City.

2.6 | Dietary starch intake measurement

Dietary starch intake information was collected in a subset of 318 children (198 with normal-weight and 120 with obesity, Table 1) from our discovery study sample (N_{total} = 764). Intake patterns were obtained through a frequency questionnaire of semi-quantitative food consumption.[30] The average daily consumption was calculated in grams of food according to the frequency reported. The consumption of macronutrients and the total energy intake for each child was determined using the database of composition food of ENSANUT 2012.[30] Finally, to determine the daily dietary starch intake, we subtracted monosaccharides and disaccharides from the total carbohydrate intake.

2.7 | Meta-analysis

To confirm the association of the CN of *AMY1A* with childhood obesity in the Mexican population, we performed a meta-analysis using individual participant data from four independent cohorts with no overlap between studies (3100 children: $N_{normal\text{-}weight}$ = 1693; $N_{obesity}$ = 1407, Table S1). Individual participant data meta-analyses are more powered than study-level meta-analyses and are considered as the gold standard in epidemiology.[31,32] A double-blind search in the PubMed database was performed by M.V.M. and A.M.B. using the following key words: ("CNV" OR "copy-number variant") AND "obesity" AND "children" AND ("Mexican" OR "Mexico") AND "*AMY1A*". Two eligible studies in the literature including 304 controls and 293 cases from five different States of Mexico[26] and 485 controls and 436 cases from Mexico City[27] were selected. We also included two unpublished studies with genotype data pertaining to *AMY1A* CNs: 277 controls and 174 cases of obesity from the current study, as well as 627 controls and 504 cases from 14 States of Mexico. In all the studies, children with normal-weight and obesity were defined by the CDC 2000 criteria.[28] Logistic regression was used to test the association between *AMY1A* CN as a continuous or categorical (tertiles) variable and childhood obesity status. The regression model was adjusted for age, sex, study and state.

2.8 | Data analysis

The normal distribution of continuous variables was tested using the Shapiro-Wilk test. For the traits that significantly deviated from normality (ie, AMY1 and AMY2 enzyme activities), a rank-based inverse normal transformation was applied and the transformed values were used in the analyses (Table S2). The original units of measure in each variable were not affected by the rank-based inverse normal transformation (Figure S1). Differences between cases and controls for continuous and categorical traits were tested with Student's *t* and Chi^2 test, respectively. The correlation between continuous traits was evaluated by Spearman's Rho. The association with continuous and categorical traits was assessed using linear and logistic regression models adjusted for age, sex and state. We created tertiles of AMY1/AMY2 serum enzymatic activities (low, medium and high) based on the raw data (Table S3). We then assessed the association between different combinations of tertiles of AMY1/AMY2 enzymatic activity, separately and together, with childhood obesity. The low AMY1, low AMY2, and alternatively the low AMY1/low AMY2 and medium AMY1/medium AMY2 enzymatic activity groups were considered as the reference groups. For the dietary starch intake, we used the residual model adjusted for energy and residuals that were taken from a regression analysis with starch intake (grams per day) as the dependent variable and total energy intake (MJ per day) as an independent variable. The interaction of starch intake with *AMY1A*/*AMY2A* CN and enzymatic activity of AMY1 and AMY2 on risk of childhood obesity was assessed using logistic regression. The variables of the main effect (*AMY1A*/*AMY2A* CN and enzymatic activity of AMY1 and AMY2) and the interaction term were entered into the model and adjusted for age and sex to evaluate the effect on obesity risk. We did not perform association studies between enzymatic activity and CN and SDS-BMI as a continuous trait. Obesity case control study designs are not compatible with these analyses and hence may result in biased estimations of the effects.[33] Based on the fact that (a) the present study is hypothesis-driven; (b) several independent research questions

are investigated; (c) a subset of these research questions have been previously tested in the literature; (d) several outcomes are not independent; applying a global Bonferroni correction across all the outcomes reduces the chance of making type I errors, but increases the chance of making type II errors.[34,35] Therefore, two-sided *P*-values <.05 were considered significant for each research question. All statistical analyses were performed with SPSS (version 22.0, IBM, Armonk, NY).

3 | RESULTS

3.1 | Characteristics of the study population

The characteristics of the 427 and 337 participants with normal-weight and obesity (discovery sample) are shown in Table 1. In the discovery sample, the average BMI in children with obesity was 7.5 kg/m^2 higher and 1.62 units higher with respect to SDS-BMI units. The obesity group contained 6.6% more boys than their normal-weight counterparts. The age was similar in the two groups.

3.2 | Correlation between serum enzyme activity of AMY1 and AMY2

A positive correlation between the continuous values of serum enzymatic activity of AMY1 and AMY2 was observed in children with normal weight (Rho = 0.173, $P = 3.1 \times 10^{-4}$) and obesity (Rho = 0.217, $P = 5.7 \times 10^{-5}$) (Figure S2).

3.3 | Association of serum enzymatic activity of AMY1 and AMY2 with childhood obesity

In children with normal weight, enzymatic activity levels of salivary and pancreatic amylase were higher by 14.3% and 10%, respectively, than in children with obesity (AMY1: 40.7 ± 20.5 vs 35.6 ± 19.4, $P = .001$; AMY2: 22.0 ± 7.0 vs 20.0 ± 6.4, $P = 5.7 \times 10^{-5}$, Table 1). Tests adjusted for age, sex and state also showed a negative association between serum enzymatic activity of AMY1 and AMY2 as a continuous variable, and childhood obesity (AMY1: N = 764, OR = 0.984 [95% confidence interval (CI95%) 0.976-0.992], $P = 4.493 \times 10^{-5}$; AMY2: N = 764, OR = 0.960 [CI95% 0.938-0.983], $P = .001$). We then created tertiles of low (<27.7 IU/L), medium (27.7-44.4 IU/L) and high (>44.4 IU/L) activity of AMY1 and low (<17.8 IU/L), medium (17.8-22.9 IU/L) and high (>22.9 IU/L) activity of AMY2 (Table S3). The global association of AMY1 enzymatic activity tertiles with childhood obesity was significant (OR = 0.716 [CI95% 0.597-0.860], $P = 3.42 \times 10^{-4}$, test adjusted for age, sex and state). Children with medium and high AMY1 enzymatic activity had a significantly lower risk of obesity when compared to children with low AMY1 enzymatic activity (OR = 0.676 [CI95% 0.473-0.968], $P = .032$ and OR = 0.509 [CI95% 0.351-0.738], $P = 3.63 \times 10^{-4}$, respectively, tests adjusted for age, sex and state). Similarly, the global association of AMY2 enzymatic activity tertiles with childhood obesity was significant (OR = 0.754 [CI95% 0.631-0.902], $P = .002$, test adjusted for age, sex and state). Children with high (but not medium) AMY2 enzymatic activity had a significantly lower risk of obesity when compared to children with low AMY2 enzymatic activity (OR = 0.769 [CI95% 0.541-1.093], $P = .143$ and OR = 0.572 [CI95% 0.400-0.819], $P = .002$, respectively, tests adjusted for age, sex and state). We then tested the association of the nine combinations of AMY1/AMY2 enzymatic activity tertiles with childhood obesity, based on their positive correlation in our sample. The global association of these categories with obesity was significant (OR = 0.901 [CI% = 0.857-0.948], $P = 5.564 \times 10^{-5}$, test adjusted for age, sex and state). Children with low-medium, medium-low, medium-high, high-low, high-medium and high-high enzymatic activity of AMY1/AMY2 had a significantly lower risk of obesity when compared to children with low-low enzymatic activity of AMY1/AMY2 (Table 2). Using children with medium-medium enzyme acivity of AMY1/AMY2 as the reference group did not sensibly change the results (Table S4).

3.4 | Correlation between CNs of *AMY1A* and *AMY2A* genes

The CN of *AMY1A* in the whole sample ranged from 2 to17. Sixty-one percent of children displayed a multiple of two copies of *AMY1A* (ie, even CN). Carriers of six copies of the *AMY1A* CN were the most frequent (18.5%) (Figure 2). In contrast, *AMY2A* CN ranged from 2 to 4 in the whole sample. Eighty-two percent of children displayed two or a multiple of two copies of *AMY2A*. Carriers of two copies of the *AMY2A* were the most common (81.4%) (Figure 2). Children with normal-weight having a multiple of two copies of *AMY1A* were more likely to have two copies of *AMY2A* (OR = 2.063 [CI95% = 1.082-3.931], $P = .028$). Similar pattern was found for children with obesity (OR = 2.251 [CI95% = 1.062-4.772], $P = .034$). We also found a significant positive correlation between CN of *AMY1A* and *AMY2A* as continuous variables in children with normal-weight (Rho = 0.179, $P = .003$, Table S5. However, this correlation was not significant in children with obesity (Rho = 0.029, $P = .707$, Table S5).

3.5 | Association between CNs of *AMY1A* and *AMY2A* genes and serum enzyme activity of salivary and pancreatic amylase

The CN of *AMY1A* and *AMY2A* were positively associated with serum enzymatic activity of salivary and pancreatic amylase, respectively, in children with normal weight (CN and enzymatic activity analyzed as continuous traits; salivary: $\beta = 4.192 \pm 0.325$, $P = 6.1 \times 10^{-19}$; pancreatic: $\beta = 3.496 \pm 0.976$, $P = 4.04 \times 10^{-4}$) and obesity (salivary: $\beta = 4.441 \pm 0.3292$, $P = 3.8 \times 10^{-20}$; pancreatic: $\beta = 6.741 \pm 1.026$, $P = 6.024 \times 10^{-10}$) (Figures S3 and S4). In order to evaluate the potential impact of four copies of *AMY2A* on the association with

TABLE 2 Association of different combinations of enzyme activity of AMY1 and AMY2 with obesity in 764 Mexican children

Enzyme activity group					
AMY1	**AMY2**	**Weight status**	**N (%)**	**Obesity**	***P***
L[a]	L[a]	Normal	42 (40)	—	—
		Obese	65 (60)		
L	M	Normal	45 (57)	0.388	**0.002**
		Obese	34 (43)	(0.213-0.708)	
L	H	Normal	37 (54)	0.606	0.140
		Obese	32 (46)	(0.311-1.179)	
M	L	Normal	54 (59)	0.469	**0.014**
		Obese	38 (41)	(0.256-0.860)	
M	M	Normal	40 (47)	0.658	0.172
		Obese	46 (53)	(0.360-1.200)	
M	H	Normal	50 (65)	0.295	$\mathbf{1.72 \times 10^{-4}}$
		Obese	27 (35)	(0.156-0.557)	
H	L	Normal	30 (52)	0.510	**0.044**
		Obese	28 (48)	(0.265-0.981)	
H	M	Normal	56 (63)	0.347	**0.001**
		Obese	33 (37)	(0.187-0.645)	
H	H	Normal	73 (68)	0.239	$\mathbf{9.436 \times 10^{-6}}$
		Obese	34 (32)	(0.127-0.450)	
Global		Normal	427 (56)	0.901	$\mathbf{5.564 \times 10^{-5}}$
		Obese	337 (44)	(0.857–0.948)	

Abbreviations: AMY1, salivary amylase; AMY2, pancreatic amylase.

Notes: Enzyme activity AMY1 (IU/L), L, low (<27.7), M, medium (27.7-44.4), H, high (>44.4). Enzyme activity AMY2 (IU/L), L, low (<17.8), M, medium (17.8-22.9), H, high (>22.9). Analysis by logistic regression, model adjusted for age, sex and state. Significant *P* values ($P < .05$) are reported in bold.

[a]Reference group in the analysis.

serum enzymatic activity of pancreatic amylase, we performed a sensitivity analysis without the four copies of *AMY2A* gene carriers. The CN of *AMY2A* was still significantly associated with serum enzymatic activity of pancreatic amylase in children with normal weight ($\beta = 4.074 \pm 1.059$, $P = 1.5 \times 10^{-4}$) and obesity ($\beta = 6.415 \pm 1.094$, $P = 2.358 \times 10^{-8}$). The cross associations of *AMY1A* CN/AMY2 enzymatic activity and *AMY2A* CN/AMY1 enzymatic activity as continuous variables were not significant in any group of study (normal weight: $P = .653$/$P = .123$; obesity: $P = .315$/$P = .931$) (Figures S5 and S6).

3.6 | Association of CN of *AMY1A* and *AMY2A* genes with obesity

The association of *AMY1A* and *AMY2A* CN analyzed as a continuous trait with childhood obesity was not significant (N = 451, OR = 0.985 [CI95% = 0.934-1.039], $P = .584$; OR = 0.854 [CI95% = 0.559-1.304], $P = .465$, respectively). Based on their distribution in the whole sample, we created tertiles of low (<6), medium (6-8) and high (>8) CN of *AMY1A* and low (<2), medium (2) and high (>2) CN of *AMY2A* (-Table S3). Again, the association of *AMY1A* and *AMY2A* with childhood obesity was not significant ($OR_{AMY1A} = 1.000472$ [CI95% = 0.850-1.178], $P = .995$; $OR_{AMY2A} = 0.848$ [CI95% = 0.543-1.323], $P = .467$). Both analyses were adjusted for age, sex, study and state. We then created a new variable resulting from the multiplication of CNs of *AMY1A* and *AMY2A*, based on their positive correlation in our sample. The association of *AMY1A*/*AMY2A* CN combinations with childhood obesity only showed a trend of association when adjusted for age, sex and state (OR = 0.827 [CI95% = 0.673-1.017], $P = .072$).

3.7 | Interaction between CN of *AMY1A* and *AMY2A* genes, enzymatic activity of salivary and pancreatic amylase, starch intake and obesity risk

In a subset of 198 and 120 children with normal-weight and obesity, we then investigated if starch intake modulates the association of CN of *AMY1A* and *AMY2A* genes, and serum enzymatic activity of AMY1 and AMY2 with childhood obesity (Table 1). Children with normal weight and obesity presented similar starch intake (g/day) (normal weight = 92.6 ± 35.7 vs obesity = 93.6 ± 37.4; $P = .815$). The CN of *AMY1A* and *AMY2A* genes did not show a significant interaction with starch intake on the risk of childhood obesity when analyzed

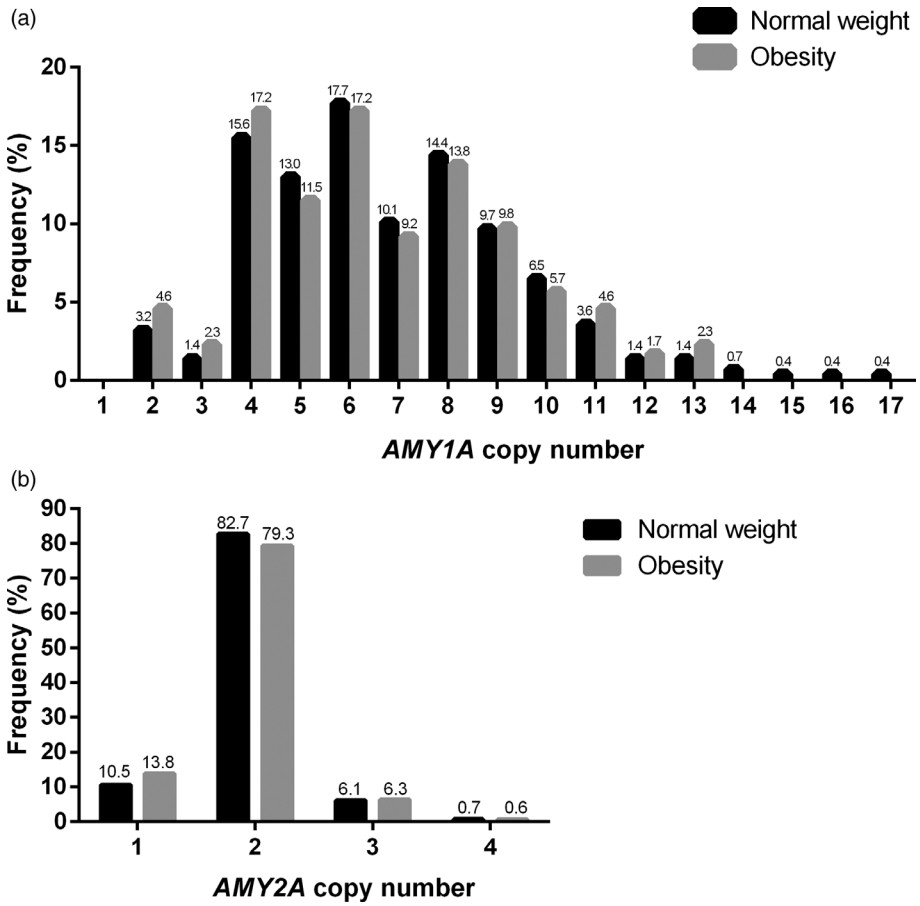

FIGURE 2 Distributions of (A) *AMY1A* and (B) *AMY2A* copy numbers in 451 Mexican children (277 with normal weight and 174 with obesity)

separately (CN and starch intake analyzed as continuous traits, tests adjusted for age and sex, Table 3). Similar nonsignificant results were observed for the *AMY1A*/*AMY2A* CN tertile combinations (Table 3). In contrast, the enzyme activity of AMY1, AMY2 displayed a significant interaction with starch intake when tested with the risk of childhood obesity (CN and starch intake analyzed as continuous traits, tests adjusted for age and sex, $P = .049$ and $P = .047$, respectively, Table 3). Similarly, the combinations of tertiles of enzyme activity of AMY1 and AMY2 showed a significant interaction with starch intake on the risk of childhood obesity ($P = .023$, Table 3). To confirm the interaction between the combination of AMY1/AMY2 enzymatic activity, starch intake and obesity, we used combinations of tertiles of enzyme activity of AMY1 and AMY2 and tertiles of starch intake (Table S3). We confirmed a significant interaction between the combinations of tertiles of enzyme activity of AMY1 and AMY2 and tertiles of starch intake ($P = .004$). Post-hoc analyses confirmed that the combinations of tertiles of AMY1 and AMY2 enzymatic activity were associated with childhood obesity in the medium and high tertiles of starch

TABLE 3 Interaction of starch intake with AMY1A/AMY2A copy numbers and enzyme activity of AMY1 and AMY2 on risk of childhood obesity

		Association	
Interaction of starch intake with:	**Normal weigh/Obesity (N)**	**OR (95% IC)**	***P***
AMY1A copy number	191/117	0.999971 (0.999-1.001)	.928
AMY2A copy number	162/89	1.001 (0.998-1.004)	.625
Combinations of *AMY1A* and *AMY2A* copy numbers (LL/LM/LH/ML/MM/MH/HL/HM/HH)	162 /89	1.00015 (0.99953-1.00077)	.645
AMY1 enzyme activity	182/115	0.999888 (0.999777-0.999999)	**.049**
AMY2 enzyme activity	182/115	0.999739 (0.999481-0.999997)	**.047**
Combinations of AMY1 and AMY2 enzyme activity (LL/LM/LH/ML/MM/MH/HL/HM/HH)	182/115	0.99990 (0.99981-0.99999)	**.023**

Abbreviations: LL, low-low; LM, low-medium; LH, low-high; ML, medium-low; MM, medium-medium; MH, medium-high; HL, high-low; HM, high-medium; HH, high-high.

Notes: Analysis by logistic regression model adjusted for age and sex. Significant *P* values ($P < .05$) are reported in bold.

intake (P = .012 and P = .006, respectively), but not in the low starch intake subgroup (P = .77) (tests adjusted for age and sex, Figure 3).

3.8 | Meta-analysis of association of the CN of *AMY1A* gene with childhood obesity in the Mexican population

Since two larger Mexican cohorts previously reported an association between *AMY1A* CN and childhood obesity,[26,27] the lack of association in our sample may be related to modest statistical power. We therefore performed a meta-analysis using individual participant data from four independent Mexican case control studies ($N_{obesity}$ = 1407, $Nnormal_{-weight}$ = 1693, Table S1). The mean CN was lower in children with obesity compared to normal weight (6.4 ± 2.6 copies vs 6.9 ± 6.4 copies, $P = 1.0 \times 10^{-6}$). A significant negative association was found between *AMY1A* CN as a continuous variable and childhood obesity (OR = 0.937 [CI95% = 0.912-0.962], $P = 2.0 \times 10^{-6}$, Table S6). We then categorized the *AMY1A* CN using tertiles (low (<5), medium (5-8) and high (>8)) based on their distribution in the whole sample (N = 3100). Again, a significant negative association between *AMY1A* CN and childhood obesity was observed (OR = 0.807 [CI95% = 0.726-0.897], $P = 7.5 \times 10^{-5}$, Table S7). Both tests were adjusted for age, sex, study and state. Sex and state, but not age and study, were significantly associated with childhood obesity in both regression models (Tables S6 and S7).

4 | DISCUSSION

We focused our research in Mexican children who are characterized by high-dietary intake of starch and high prevalence of obesity. We evidenced (a) a positive correlation between AMY1/AMY2 serum enzymatic activity in children with normal weight and obesity; (b) a negative association between the serum enzymatic activity of AMY1, AMY2 and their combinations and childhood obesity in individuals eating medium/high amount of starch; 3) a contrasted pattern of correlation between *AMY1A* and *AMY2A* CNs in children with normal weight and obesity; (d) a positive association between *AMY1A*/*AMY2A* CNs and AMY1/AMY2 serum enzymatic activity in children with normal weight and obesity; (e) a trend of association between *AMY1A*/*AMY2A* CN combinations and childhood obesity; (f) a compelling association between *AMY1A* CN and childhood obesity in a meta-analysis of >3000 participants.

Our results are consistent with the existing literature. As an illustration, the negative association between *AMY1A* CN and childhood obesity observed in our meta-analysis of Mexican participants supported findings from two previous studies in the Mexican population.[26,27] While most of our findings (points 1-5 as cited above) have been evidenced for the first time in the Mexican population, they are in line with previous reports of European children and adults.[13,16,17,25] Carpenter et al evidenced a correlation between *AMY1A*/*AMY2A* CNs in European adults.[16] Similarly, Falchi et al reported a positive association between *AMY1A*/*AMY2A* CNs and AMY1/AMY2 serum enzymatic activity in French individuals with morbid obesity.[13] Strong negative associations between AMY1 or AMY2 enzymatic activity and BMI were also reported by Bonnefond et al in the French population.[17] Additionally, Rukh et al evidenced that dietary starch intake modifies the relation between *AMY1A* CN and BMI in Swedish adult participants.[25] Our data contributes to unveil a biological mechanism that results in high genetic predisposition to obesity in Mexican children. A genetically determined low/high salivary and pancreatic amylase activity can increase/decrease the risk of obesity in children with a medium/high-starch diet. The association between deficient digestion of starch, as induced by low enzymatic activity of AMY1 and AMY2 amylases, and obesity may seem counter-intuitive at a first glance. However, recent data suggest that it could represent a case of intestinal dysbiosis given the contribution of oligosaccharides as a substrate to the gut microbiota.[27] This is illustrated by the significant association between *AMY1A* CN and *Prevotella* bacteria abundance in the gut microbiome of Mexican children and adults reported by Leon-Mimila et al.[27] Considering the important role of gut microbiome

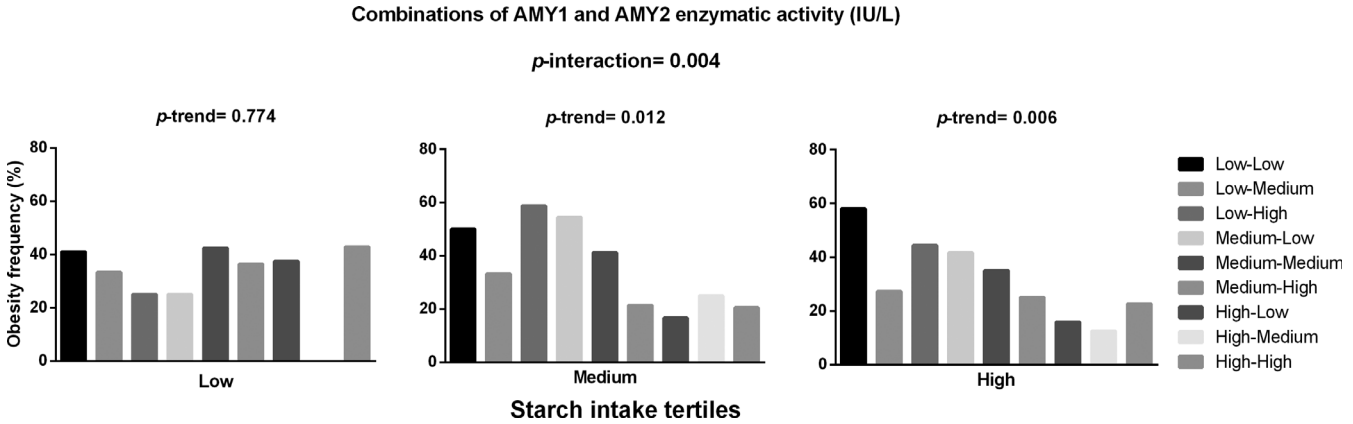

FIGURE 3 Association of different combinations of tertiles of enzyme activity of AMY1 and AMY2 with obesity risk in tertiles of starch intake. $N_{Low\ starch\ intake}$ = 105; $N_{Medium\ starch\ intake}$ = 108; $N_{High\ starch\ intake}$ = 105. Data as frequency of obesity. *P*-interaction and *P*-trend by logistic regression model adjusted for age and sex

composition in the regulation of energy balance,[36] deciphering the impact of *AMY1A/AMY2A* CNs/enzymatic activity of AMY1 and AMY2 on the whole gut microbiome diversity and on bacterial amylase activity in feces represents an important direction of research for the future. Our study provides a compelling genetic link between carbohydrate metabolism and risk of childhood obesity in the Mexican population. This finding provides insight into original biological mechanisms underlying obesity, as well as a rationale for the investigation of innovative obesity treatments based on the manipulation of digestive enzyme levels.[13]

Our study has several strengths. This is the first report that describes the associations of AMY2A CN with AMY2 enzymatic activity and AMY1/AMY2 enzymatic activity with obesity in Mexican children. We focused our research on Mexican children, a traditionally under-investigated but highly relevant population characterized by high-starch intake and high prevalence of obesity. We used innovative technologies and sophisticated analyses for *AMY1A/AMY2A* CN, serum enzymatic activity of AMY1 and AMY2, starch intake consumption and childhood obesity, and our study can therefore be considered as the most exhaustive report to date in the Mexican population.[26,27] Our study displays a lower risk of obesity in the high-high AMY1/AMY2 enzymatic activity subgroup. Our results further elucidate how these effects can be totally blunted by a low-starch diet.

Nevertheless, our study also presents several limitations. We are aware that our experimental design was modestly powered for a subset of analyses. We also acknowledge that we used liberal cut-offs for significance (two-sided $P < .05$), and we strongly recommend that novel associations should be replicated in independent Mexican samples. We did not study Mexican adults and did not provide data on gut microbiome and bacterial amylase enzymatic activity. Lastly, we combined CN data genotyped by different methods in our meta-analysis of association between *AMY1A* CN and childhood obesity. While there is no doubt that the *AMY1A/AMY2A* CN detection accuracy varies depending on the laboratory method used, the absence of between-study heterogeneity in our meta-analysis suggests that the impact of methodological bias, at least with respect to the *AMY1A* CN, may be minimal.[14,17]

In conclusion, our data suggests that genetically determined low/high salivary and pancreatic amylase activity can increase/decrease the risk of obesity by two-times in a case control Mexican pediatric population, with the effect being blunted by a low- dietary intake of starch. Our study contributes to unveil a biological mechanism that predisposes to childhood obesity, and paves the way of future nutrigenomic interventions based on dietary recommendations adapted to specific genetic/biomarker profiles in Mexican children.

CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article were reported.

ACKNOWLEDGEMENTS

M.V.M, M.C. and D.M. designed the study; M.V.M, A.M.B., T.S., J.P.R., D.L.M., M.K.K., M.C. and D.M. conducted research; M.V.M, A.M.B. and D.M. analyzed data; M.V.M., A.M.B. and D.M. wrote the manuscript; M.V.M., A.M.B. and D.M. designed the tables and figures. T.S., J.P.R., D.L.M., M.K.K. and M.C. critically reviewed the manuscript for important intellectual content; M.V.M, M.C. and D.M. had primary responsibility for final content. All authors read and approved the final manuscript. We are grateful to all participants involved in this study. We would like to acknowledge Aracely Méndez-Padron (Instituto Mexicano del Seguro Social [IMSS], Mexico) and Anila Qasim (McMaster University, Canada) for the technical assistance. We would like to thank Dr. Paola León-Mimila and Pr. Samuel Canizales-Quinteros for assistance in retrieving data for the meta-analysis. We would like to thank Dr. Jennifer Stearns for helpful discussions. This work was supported by grants from the Mexican Institute of Social Security under the program of Priority Health Topics 2017 (Grant No. FIS/IMSS/PROT/PRIO/17/062). MVM (Ciencias Médicas Odontológicas y de la Salud PhD program from Universidad Nacional Autónoma de México) and DLM (Ciencias Biomédicas PhD program from Universidad Autónoma de Guerrero) were supported by PhD fellowships from the Consejo Nacional de Ciencia y Tecnología (CONACYT) and IMSS (Mexico). In addition, MVM received a travel award from the IMSS, Mexico. D.M. is supported by a Canada Research Chair in Genetics of Obesity.

ORCID

Miguel Cruz https://orcid.org/0000-0001-9985-6172
David Meyre https://orcid.org/0000-0003-4850-7444

REFERENCES

1. Shamah-Levy T, Cuevas-Nasu L, Gomez-Acosta LM, et al. Effects of SaludArte program in feeding and nutrition components in school children in Mexico city. *Salud Publica de Mexico*. 2017;59(6):621-629.
2. Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *Int J Obes*. 2011;35(7):891-898.
3. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the global burden of disease study 2013. *Lancet*. 2014;384(9945):766-781.
4. Pigeyre M, Yazdi FT, Kaur Y, Meyre D. Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. *Clin Sci*. 2016;130(12):943-986.
5. Stern D, Piernas C, Barquera S, Rivera JA, Popkin BM. Caloric beverages were major sources of energy among children and adults in Mexico, 1999-2012. *J Nutr*. 2014;144(6):949-956.
6. Aceves-Martins M, Llaurado E, Tarro L, Sola R, Giralt M. Obesity-promoting factors in Mexican children and adolescents: challenges and opportunities. *Glob Health Action*. 2016;9:29625.
7. Wardle J, Boniface D. Changes in the distributions of body mass index and waist circumference in English adults, 1993/1994 to 2002/2003. *Int J Obes*. 2008;32(3):527-532.
8. Stryjecki C, Alyass A, Meyre D. Ethnic and population differences in the genetic predisposition to human obesity. *Obes Rev*. 2018;19(1):62-80.
9. Llewellyn CH, Trzaskowski M, Plomin R, Wardle J. Finding the missing heritability in pediatric obesity: the contribution of genome-wide complex trait analysis. *Int J Obes*. 2013;37(11):1506-1509.
10. Yang J, Bakshi A, Zhu Z, et al. Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nat Genet*. 2015;47(10):1114-1120.

11. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461(7265):747-753.
12. Walters RG, Coin LJ, Ruokonen A, et al. Rare genomic structural variants in complex disease: lessons from the replication of associations with obesity. *PloS One*. 2013;8(3):e58048.
13. Falchi M, El-Sayed MJS, Takousis P, et al. Low copy number of the salivary amylase gene predisposes to obesity. *Nat Genet*. 2014;46(5):492-497.
14. Usher CL, Handsaker RE, Esko T, et al. Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. *Nat Genet*. 2015;47(8):921-925.
15. Yong RY, Mustaffa SB, Wasan PS, et al. Complex copy number variation of AMY1 does not associate with obesity in two east Asian cohorts. *Hum Mutat*. 2016;37(7):669-678.
16. Carpenter D, Dhar S, Mitchell LM, et al. Obesity, starch digestion and amylase: association between copy number variants at human salivary (AMY1) and pancreatic (AMY2) amylase genes. *Hum Mol Genet*. 2015;24(12):3472-3480.
17. Bonnefond A, Yengo L, Dechaume A, et al. Relationship between salivary/pancreatic amylase and body mass index: a systems biology approach. *BMC Med*. 2017;15(1):37.
18. Pinho S, Padez C, Manco L. High AMY1 copy number protects against obesity in Portuguese young adults. *Ann Hum Biol*. 2018;45(5):435-439.
19. Viljakainen H, Andersson-Assarsson JC, Armenio M, et al. Low copy number of the AMY1 locus is associated with early-onset female obesity in Finland. *PloS One*. 2015;10(7):e0131883.
20. Marcovecchio ML, Florio R, Verginelli F, et al. Low AMY1 gene copy number is associated with increased body mass index in Prepubertal boys. *PloS One*. 2016;11(5):e0154961.
21. Venkatapoorna CMK, Ayine P, Parra EP, et al. Association of salivary amylase (AMY1) gene copy number with obesity in Alabama elementary school children. *Nutrients*. 2019;11(6). pii: E1379-1388.
22. Sayago-Ayerdi SG, Tovar J, Zamora-Gasga VM, Bello-Perez LA. Starch digestibility and predicted glycaemic index (pGI) in starchy foods consumed in Mexico. *Star*. 2013;66(1–2):91-101.
23. Sayago-Ayerdi SG, Tovar J, Osorio-Diaz P, Paredes-Lopez O, Bello-Perez LA. In vitro starch digestibility and predicted glycemic index of corn tortilla, black beans, and tortilla-bean mixture: effect of cold storage. *J Agric Food Chem*. 2005;53(4):1281-1285.
24. Perry GH, Dominy NJ, Claw KG, et al. Diet and the evolution of human amylase gene copy number variation. *Nat Genet*. 2007;39(10):1256-1260.
25. Rukh G, Ericson U, Andersson-Assarsson J, Orho-Melander M, Sonestedt E. Dietary starch intake modifies the relation between copy number variation in the salivary amylase gene and BMI. *Am J Clin Nutr*. 2017;106(1):256-262.
26. Mejia-Benitez MA, Bonnefond A, Yengo L, et al. Beneficial effect of a high number of copies of salivary amylase AMY1 gene on obesity risk in Mexican children. *Diabetologia*. 2015;58(2):290-294.
27. Leon-Mimila P, Villamil-Ramirez H, Lopez-Contreras BE, et al. Low salivary amylase gene (AMY1) copy number is associated with obesity and gut Prevotella abundance in Mexican children and adults. *Nutrients*. 2018;10(11). pii: E1607-1619.
28. Kuczmarski RJ, Ogden CL, Guo SS, et al. CDC growth charts for the United States: methods and development. *Vital Health Stat*. 2000;2002(246):1-190.
29. Junge W, Wortmann W, Wilke B, et al. Development and evaluation of assays for the determination of total and pancreatic amylase at 37 degrees C according to the principle recommended by the IFCC. *Clin Biochem*. 2001;34(8):607-615.
30. Gaona-Pineda EB, Mejia-Rodriguez F, Cuevas-Nasu L, Gomez-Acosta LM, Rangel-Baltazar E, Flores-Aldana ME. Dietary intake and adequacy of energy and nutrients in Mexican adolescents: results from Ensanut 2012. *Salud Publica de Mexico*. 2018;60(4):404-413.
31. Chalmers I. The Cochrane collaboration: preparing, maintaining, and disseminating systematic reviews of the effects of health care. *Ann NY Acad Sci*. 1993;703:156-163. discussion 163-155.
32. Ehtesham S, Qasim A, Meyre D. Loss-of-function mutations in the melanocortin-3 receptor gene confer risk for human obesity: a systematic review and meta-analysis. *Obes Rev*. 2019;20(8):1085-1092.
33. Wang J, Shete S. Estimation of odds ratios of genetic variants for the secondary phenotypes associated with primary diseases. *Genet Epidemiol*. 2011;35(3):190-200.
34. Feise RJ. Do multiple outcome measures require p-value adjustment? *BMC Med Res Methodol*. 2002;2:8.
35. Leon AC, Heo M, Teres JJ, Morikawa T. Statistical power of multiplicity adjustment strategies for correlated binary endpoints. *Stat Med*. 2007;26(8):1712-1723.
36. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444(7122):1027-1031.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Vázquez-Moreno M, Mejía-Benítez A, Sharma T, et al. Association of *AMY1A/AMY2A* copy numbers and AMY1/AMY2 serum enzymatic activity with obesity in Mexican children. *Pediatric Obesity*. 2020;e12641. https://doi.org/10.1111/ijpo.12641

APPENDIX A: | AUTHOR'S APPENDIX

National Obesity Network Mexico.

Baja California Sur State: Andrea S. Álvarez-Villaseñor; Kelly G. Acosta; Raquel Flores-Torrecillas; Uriel Flores-Osuna; Mariell G. García-Avilés.

Campeche State: Roxana del S. González-Dzib.

Chihuahua State: René A. Gameros-Gardea.

México State: María L. Pizano-Zárate; María del S. Romero-Figueroa; Jorge A. Núñez-Hernández; Verónica de León-Camacho;

Mexico City: Roberto Karam-Araujo; Perla Corona-Salazar; Adán Valladares-Salgado, Fernando Suarez-Sánchez; Jaime Gómez-Zamudio; Eugenia Flores-Alfaro.

Guanajuato State: Gloria P. Sosa-Bustamante; Arturo Reyes-Hernández; Catalina Peralta-Cortázar; Emmanuel G. Martínez-Morales; Luz V. Díaz de León-Morales; Irma L. del C. González-González; Arturo M. Reyes-Sosa; Sonia Lazcano-Bautista; Marcela Martínez-Chávez; Fátima D. González-Serrano; Alma N. Rodríguez-Alfaro; Blanca O. Murillo-Ortiz.

Hidalgo State: María G. Arteaga-Alcaraz; Nandy García-Silva; Moisés Herrera-Lemus; Gress M. Gómez-Arteaga.

Michoacán State: Anel Gómez-García; Martha V. Urbina-Treviño; Diana C. Villalpando-Sánchez; Cleto Álvarez-Aguilar.

Nayarit: Ramón E. Jiménez-Arredondo.

Monterrey State: Martha I. Dávila-Rodríguez; Francisco González-Salazar; Laura H. de la Garza-Salinas.

Oaxaca State: Aleyda Pérez-Herrera.

Puebla State: Socorro Méndez-Martínez; Jorge Martínez-Torres; Elizabeth Méndez-Fernández; Víctor A. Segura-Bonilla; Mariana Gutiérrez-Hernández.

Querétaro State: Lilia S. Gallardo-Vidal; Leticia Blanco-Castillo; José J. García-González.

Sinaloa State: Julio M. Medina-Serrano; Adrián Canizalez-Román.

Sonora State: Cruz M. López-Morales; Jaime G. Valle-Leal.

Tamaulipas State: Martin Segura-Chico; Rafael Violenté-Ortiz; Verónica Fernández-Jiménez; Norma A. Sánchez-Hernández.