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Genetic determinants of BMI from early childhood to adolescence: the Santiago Longitudinal Study

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Summary

Background: While the genetic contribution to obesity is well established, few studies have examined how genetic variants influence standardized body mass index *Z*-score (BMIz) in Hispanics/Latinos, especially across childhood and adolescence.

Objectives: We estimated the effect of established BMIz loci in Chilean children of the Santiago Longitudinal Study (SLS).

Methods: We examined associations with BMIz at age 10 for 15 loci previously identified in European children. For significant loci, we performed association analyses at ages 5 and 16 years, for which we have smaller sample sizes. We tested associations of unweighted genetic risk scores (GRSs) for previously identified tag variants (GRS_EUR) and from the most significant variants in SLS at each locus (GRS_SLS).

Results: We generalized five variants at age 10 ($P < 0.05$ and directionally consistent), including rs543874 that reached Bonferroni-corrected significance. The effect on BMIz was greatest at age 10 for all significant loci, except *FTO*, which exhibited an increase in effect from ages 5 to 16. Both GRSs were associated with BMIz ($P < 0.0001$), but GRS_SLS explained a much greater proportion of the variation (13.63%).

Conclusion: Our results underscore the importance of conducting genetic investigations across life stages and selecting ancestry appropriate tag variants in future studies for disease prediction and clinical evaluation.

Keywords: BMI, genetic risk score, Latin America, obesity.

Introduction

Obesity is a leading risk factor for cardiovascular disease, and its global prevalence has more than doubled since the 1980s (1). Latin Americans are at high risk (2,3), with rapid expansion of obesity and cardiovascular disease in the context of accelerating urbanization. This study's participants are from Chile, where dramatic socioeconomic improvement accompanied by changes in diet, physical activity and sociocultural environment has resulted in obesity prevalence among the highest in the world (4,5). Indeed, according to Chile's Third National Health Survey, 74.2% of individuals over 15 years of age are currently overweight or obese (6). Also, Hispanic/Latinos transition from normal weight to obesity at younger ages than age-matched members of other race-ethnic groups (7).

Genetic factors are a well-established contributor to obesity risk (8,9). Although, most genome-wide association studies (GWASs) of obesity-related traits have largely been in adult, European descent (EUR) populations (8). Recent studies suggest that genetic effects on obesity-related phenotypes, like body mass index (BMI), may differ across life stages (10,11). To date, few genetic studies have focused on the effects of genetics across childhood (9,11,12), or Hispanic/Latinos (12), including Chileans, although they shoulder a high burden of obesity. These observations highlight the need to characterize the relevance of established genetic loci from infancy into adulthood. Also, genetic risk scores (GRSs) are used to summarize phenotype–genotype relationships and for prediction of disease and clinical evaluation (13) and have been used to explore the genetic contribution to weight,

weight gain and BMI *Z*-score (BMIz) in infants and young EUR children less than 5 years old (14,15). Yet the applicability and appropriateness of using EUR tag variants in GRS constructed in ancestrally diverse populations has not been widely evaluated.

Using data available in the Santiago Longitudinal Study (SLS) of Chilean children, we aimed to (i) estimate the association between established BMIz genetic risk markers first identified in European children; (ii) determine if genetic effects for significantly associated single nucleotide polymorphisms (SNPs) differ by age in our SLS cohort; and (iii) investigate the association of BMIz with cumulative GRS derived using EUR tag SNPs and potential SLS population-specific tag SNPs.

Materials and methods

Santiago Longitudinal Study

Santiago Longitudinal Study participants were recruited during infancy in Santiago, Chile, and have been followed through childhood and adolescence into early adulthood. The parent nutrition study, which began as an iron deficiency anaemia (IDA) preventive trial, has been described elsewhere (16). Briefly, from 1991 to 1996, 1798 infants with no major health problems, born at term and weighing ≥3.0 kg, participated in a randomized trial of iron supplementation to prevent IDA, a neurophysiology study of those with IDA, and non-anaemic controls. Children were followed up after infancy at 5, 10 and 16 years of age (17,18). The children's families were generally lower/middle class, literate and of mixed American Indian and Spanish descent (16,19). All parents of participants provided informed consent at all visits, and participants themselves provided assent at age 16. The current study has been approved by Institutional Review Boards at the University of California at San Diego, University of Michigan, University of North Carolina at Chapel Hill, Geisinger Health, and Institute of Nutrition and Food Technology, University of Chile.

Anthropometry and laboratory assessment

Childhood weight and height were measured in duplicate by a study nurse or physician using standard techniques at assessment at 5, 10 and 16 years old. Weight was measured to the nearest 0.1 kg using a SECA scale and height to the nearest 0.1 cm using a Holtain stadiometer. BMI was calculated as weight (kg)/height^2 (m^2) and then transformed into *Z*-scores relative to CDC anthropometric reference data (2007–2010) (20). These BMIz were compared with those estimated using the World Health Organization (BMIzWHO) reference data and the UK (BMIzUK) reference data for all three ages and for boys, girls and combined sexes. Overall, the correlation (R^2) reported was ≥98% (BMIzCDC vs. BMIzUK) and ≥94% (BMIzCDC vs. BMIzWHO), respectively, and highest among age 10 group (99%) for both comparisons (Fig. S1). As estimated BMIz was highly concordant across all panels, all results presented herein are for BMIzCDC.

Genotypic data and single nucleotide polymorphism selection

We examined associations for 15 loci previously associated with BMIz in European children aged 2 to 10 years old from Felix *et al*. (9). For one of the 15 loci, the previously identified tag variant was unavailable in our genotype data, and no proxy SNP was available to test ($R^2 > 0.9$). Therefore, we evaluated the association of BMIz at age 10 with 14 SNPs previously associated with BMIz in EUR children aged 2 to 10 years old (9) (Table 1). Illumina Infinium Multi-Ethnic Global-8 array was utilized to acquire genotypic data in unrelated participants using whole blood samples collected at the 16-year follow-up visit. Quality control included filtering on individual call rate >90%, checking for gender mismatch, relatedness and ancestry outliers. All variants had a minor allele frequency >0.005, Hardy–Weinberg equilibrium ($P > 1 \times 10^{-7}$) and call rate >95%.

Statistical analysis

For all SNPs positioned within the 1-MB interval (+/− 500 kb from known EUR tag SNP) of 15 established BMIz loci, we conducted linear regression of BMIz at age 10 (the age with the largest sample size). Analyses were conducted in PLINK 1.07 (21) assuming an additive genetic model, adjusted for population substructure using principal components (PCs) calculated from genome-wide data in EIGENSTRAT (22). To correct for multiple testing, we considered SNPS that displayed a Bonferroni-corrected $P < 0.0017$ as significant. For each variant that displayed a nominally significant association ($P < 0.05$) with BMIz at age 10, we further examined the effect of that SNP at ages 5 and 16. Additionally, sensitivity analysis was performed on our data after adjusting for random clinical trial (RCT) arm of the parent infant study based on iron supplementation ('high-iron' – 12 mg L^{-1}, 'low-iron' – 2.4 mg L^{-1} or 'no-iron'). The RCT variable was not a significant predictor of BMIz. Thus, all results presented herein are without adjustment for RCT.

Table 1 Summary of association results for our Santiago Longitudinal Cohort Study (SLS) compared with previously identified effects in European descent children as reported in Felix *et al*. (9)

						SLS				Felix *et al*.
Nearest gene	SNP	CHR	BP	EA/OA	EAF	Age	β	SE	*P*	β (+/− based on EA)
Significant loci										
SEC16B	rs543874	1	177889480	G/A	0.27	5	0.126	0.065	0.055	+
						10	0.185	0.052	***4.3E-04***	
						16	0.164	0.067	**0.015**	
						Change	0.0014	0.0019	0.467	
ADCY3	rs11676272	2	25141538	G/A	0.34	5	0.064	0.061	0.297	+
						10	0.099	0.049	**0.043**	
						16	0.061	0.063	0.331	
						Change	−0.0005	0.0018	0.795	
FAIM2	rs7132908	12	50263148	A/G	0.22	5	0.033	0.070	0.639	+
						10	0.116	0.058	**0.046**	
						16	0.059	0.072	0.410	
						Change	0.0002	0.0012	0.936	
OLFM4	rs12429545	13	54102206	A/G	0.32	5	0.090	0.059	0.133	+
						10	0.100	0.048	**0.037**	
						16	0.042	0.062	0.496	
						Change	−0.0017	0.0017	0.324	
FTO	rs1421085	16	53800954	C/T	0.22	5	0.060	0.070	0.391	+
						10	0.119	0.055	**0.032**	
						16	0.177	0.071	**0.014**	
						Change	0.0036	0.0020	0.075	
Non-significant loci										
TNNI3K	rs12041852	1	75003500	A/G	0.35	10	0.024	0.049	0.628	−
GPR61	rs7550711	1	110082886	C/T	0.98	10	0.079	0.219	0.715	−
TMEM18	rs4854349/ rs4854348*	2	647760	G/A	0.87	10	0.102	0.072	0.159	+
GNPDA2	rs13130484	4	45175691	C/T	0.61	10	0.077	0.048	0.112	−
TFAP2B	rs987237	6	50803050	G/A	0.37	10	0.088	0.051	0.087	+
ELP3	rs13253111/ rs7821358*	8	28060690	A/G	0.59	10	0.043	0.048	0.374	+
LMX1B	rs3829849/ rs62578126*	9	129375338	T/C	0.23	10	0.063	0.058	0.272	+
RAB27B	rs8092503/ rs8085272*	18	52481583	A/G	0.29	10	−0.001	0.052	0.985	−
MC4R	rs6567160	18	57829135	C/T	0.12	10	0.005	0.071	0.943	+

*Linkage disequilibrium (R^2) between tag and proxy SNPs ranged from 0.94 to 1.0.
β, beta coefficient; BP, base pair position; CHR, chromosome; EA, effect allele; EAF, effect allele frequency; OA, other allele; SE, standard error; SNP, single nucleotide polymorphism.
Bold represents all nominally significant associations ($P < 0.05$); italics represents associations that meet significance for Bonferroni-correction ($P < 0.0017$).

Genetic effects on body mass index *Z*-score across age

For variants that are nominally associated with BMIz at age 10, we performed two additional analyses to evaluate the effect of these variants on BMIz across age. First, we tested to see if there was a significant change in effect across ages 5, 10 and 16. For this test, we performed a Spearman's rank correlation in STATA 15.1 with nominally significant BMIz-associated SNPs across age groups 5, 10 and 16 to assess a significant trend in SNP effect size across age ($P < 0.05$). As sample size at ages 5 and 16 was lower than at age 10 (N = 577, 543 and 770,

respectively), we did not formally test for heterogeneity effects between ages and instead focus our analysis on only a monotonic trend in effect across ages. Second, we evaluated the effect of each of these loci on change in BMIz as measured by the slope of BMIz score change across ages 5 to 16. Using linear mixed model regression with an unstructured variance/covariant matrix and restricted maximum likelihood estimation, BMIz was regressed with visit age as both fixed and random effects to derive the slope of adiposity change based on best linear unbiased predictor. Only individuals with at least two measures of BMI were included in this analysis (N = 574). The resulting change in BMIz was then used as the outcome variable in the genetic association analysis, assuming an additive genetic model and adjusting for population substructure using PCs as before. These analyses were carried out using STATA v. 15.1.

Conditional analyses

We identified the most significant SNP positioned within the 1-MB interval (+/− 500 kb from known EUR tag SNP) of 15 established EUR GWAS findings for BMIz at age 10. For each locus where the most significant SNP identified differed from the published SNP, we performed exact conditional analysis in PLINK 1.07 (21) to determine whether or not the new SNP was independent of the established association signal (e.g. whether this SNP represented a secondary signal in the known locus or was a better tag SNP for the known locus). Signals were considered attenuated if the beta decreased by more than 10% and/or did not display nominal significance following conditional analysis.

Genetic risk score

To explore the cumulative effects of these 15 genetic regions on childhood obesity risk, we calculated GRS to estimate the variance explained in BMIz. We estimated unweighted simple-count GRS in three ways: (i) GRS_EUR: by summing the number of BMIz increasing risk alleles from the 14 known GWAS SNPs; (ii) GRS_SLS: by summing the number of BMIz increasing risk alleles from the top SNPs in the 15 loci in our SLS cohort; and (iii) GRS_ALL: by summing the number of independent BMIz increasing alleles across all 15 loci, which included the top variant at each locus and an additional 12 EUR tag SNPs that were independent of the top 15 following conditional analyses. As the true effect size and relative significance of each variant used in our GRS estimations are not well established, we have chosen to focus on unweighted GRS (GRS ranges from 0 to 28 for GRS_EUR, 30 for GRS_SLS and 54 for GRS_ALL). Multiple linear regressions of BMIz and GRSs were performed adjusting for PCs to control for population stratification in STATA 15.1.

Results

Summary statistics

Descriptive statistics are given in Table S1 for measurements taken at ages 5, 10 and 16 (N = 577, 770 and 545, respectively; 48% girls). The mean weight and height in boys is slightly higher compared with girls at ages 5 and 16. However, BMIz was lower in girls compared with boys at ages 5 and 10 but higher by age 16. On the contrary, obesity (≥95th BMI percentile) prevalence was higher in girls compared with boys at ages 5 and 16 (22.7%, 14.3% vs. 19.8%, 13.8%) but lower at age 10 (14.6% vs. 18%); however, overall obesity rate decreased from age 5 (21.2%), age 10 (16.3%) to age 16 (14.1%). In general, from ages 5 to 16, BMIz and prevalence of obesity decreased for both girls and boys as age increased relative to the CDC reference panel (Table S1).

Single nucleotide polymorphism association analysis

For one of the 15 loci, the previously identified tag variant was unavailable in our genotype data, and no proxy SNP was available to test ($R^2 > 0.9$). Therefore, we evaluated the association of BMIz at age 10 with 14 SNPs previously associated with BMIz in EUR children aged 2 to 10 years old (9) (Table 1). Ten of the 14 SNPs were directionally consistent with previous findings, of which five (near *SEC16B*, *FTO*, *OLFM4*, *ADCY3* and *FAIM2*) reached nominal significance ($P < 0.05$), including one (rs543874, near *SEC16B*) that reached Bonferroni-corrected significance ($P < 0.0017$). While there is a lack of consensus in the field on the appropriate significance threshold for interrogation of established loci for replication/generalization, our study findings support generalization of these loci to our Chilean population. However, larger sample sizes will be needed to reach convincing genome-wide significant statistical evidence.

Genetic effect of body mass index *Z*-score across age

To determine if the effect of nominally significant SNPs varied across ages 5, 10 and 16, we also examined the associations with BMIz for participants with measurements taken at ages 5 and 16 in addition to age

10 (Table 1, Fig. S2). For all five loci, the effects were directionally consistent across age, but the magnitude of the effect varied across age with a lower effect on BMIz at both ages 5 and 16 for all loci except *FTO*, which displayed a significant increase in effect across increasing age ($P_{trend} < 0.0001$). Of the five loci, two reached nominal significance at age 16 (rs543874 near *SEC16B* and rs1421085 near *FTO*) (Fig. S3), but none reached nominal significance at age 5. The per allele effect of the *SEC16B* locus was greatest at age 10 ($\beta = 0.185$, $P = 4.3E-04$) compared with both ages 5 ($\beta = 0.126$, $P = 0.055$) and 16 ($\beta = 0.164$, $P = 0.015$). Although BMIz in our Chilean cohort is higher among 5-year-olds relative to CDC reference population, there does not appear to be a strong genetic component to this.

We assessed the association of the same five SNPs on change in BMIz across ages 5 to 16 (Table 1) but did not identify any significant associations ($P > 0.05$). Among the SNPs tested, rs1421085 near FTO displayed the largest effects on BMIz change ($\beta = 0.004$, $P = 0.075$).

Aggregated genetic risk for body mass index *Z*-score

We identified the most significant SNP positioned within the 1-MB interval (+/− 500 kb from known EUR tag SNP) at each of the established 15 loci for BMIz measured at age 10 (Table 2). At each of the 15 loci, we identified a more significantly associated SNP in our SLS cohort than the tag SNP or proxy SNP for EUR children. Of these 15, seven were in low linkage disequilibrium with the previous tag SNP ($R^2 < 0.2$ and D' < 1.0 in the Central European reference panel [CEU]), one tag SNP was unavailable in our data and two were unavailable in the CEU reference data. All 15 SNPs displayed a nominally significant association with BMIz ($P < 0.05$), including five that displayed Bonferroni-corrected statistical significance ($P < 0.0017$, 0.05/29 variants). We performed exact conditional analysis for our most significant variants conditioning on the known EUR tag SNP. For all 14 loci where a EUR tag SNP or proxy SNP was available, our SLS tag SNP remained nominally significant following conditional analysis ($P_{conditional} < 0.05$), and four of the five remained significant after multiple test correction ($P_{conditional} < 0.0017$). However, for two SNPs (rs200787218 near SEC16B and rs138716876 near ADCY3), the estimated effect of the SLS SNP was somewhat attenuated ($\beta_{conditional} < 0.9*\beta$) after conditional analysis, indicating that these SNPs are likely the same association signal as those identified in Felix *et al*. (9).

Three GRSs were constructed using the tag SNPs from Felix *et al*. (GRS_EUR, 14 SNPs), the most significant SNP for each locus (GRS_SLS, 15 SNPs) and the GRS_SLS plus independent SNPs from GRS_EUR (GRS_ALL, 27 SNPs), as highlighted in Table 2. All three GRS were significantly associated with BMIz at age 10 ($P < 0.017$, 0.05/3 GRS); however, the GRS_SLS was the most significant ($\beta = 0.161$, $P = 1.4 \times 10^{-26}$) and explained the greatest proportion of variation in BMIz (13.63%) (Table S2). Although these estimates of variance explained are likely inflated due to winner's curse (upward bias in effect estimates of novel loci), it is still very apparent that these population-specific tag SNPs represent a marked improvement of the genetic effects of these loci on BMIz for our Chilean cohort. Additionally, we observe a difference of 0.32 BMIz units between individuals with the median number of BMIz-increasing alleles (17 alleles) and those in the upper quintile (>19 BMIz-increasing alleles) (Fig. 1).

Discussion

The discovery of genetic mechanisms influencing adiposity-related traits has the potential to identify important pathways for disease prediction and treatment (23). Yet, the bulk of similar research has focused on homogeneous middle-aged adults, with very few genetic studies on ancestrally diverse, admixed children (24). By examining 15 established EUR childhood BMI variants in the SLS Chilean children, we found that several of the SNP-BMIz associations were directionally consistent with previous findings, and five (near *SEC16B*, *FTO*, *OLFM4*, *ADCY3* and *FAIM2*) reached nominal significance with age-specific effect sizes exhibiting a lower effect on BMIz at both ages 5 and 16 for all loci except *FTO*, which displayed a significant increase in effect across increasing age. Further, we find that potential SLS-specific tag SNPs represent a marked improvement over EUR tag SNPs in explaining variation in BMIz for our admixed Chilean cohort.

Previous studies of the *FTO* locus have suggested distinct effects on BMI across the life course (25,26). While cross-sectional studies have indicated that effects across age may be due to cohort effects (27), other studies highlight associations between FTO and longitudinal BMI measures across childhood and into adulthood (25,26). Both rs1558902 and rs9939609 in *FTO* have been associated with change in BMI in EUR-descent individuals aged 11–20 years (28) and with change in BMI for a meta-analysis, which included Hispanic/Latino children living in the USA (11). In our Chilean paediatric population, we

Table 2 Association results of BMIz with EUR tag SNPs compared with population-specific tag SNPs selected from the 1-MB interval of these known obesity loci in SLS

		EUR tag SNPs			R^2		D'	Population-specific tag SNPs							Conditional analyses[#]		
Nearest gene	CHR	SNP/proxy	Included in GRS	Distance	SLS	CEU	CEU	SNP	Included in GRS	EA/OA	EAF	β	SE	P	β	SE	P
TNNI3K	1	rs12041852	EUR, ALL	−174 105	0.008	0.042	1	rs61790698	SLS, ALL	G/A	0.047	0.239	0.109	**0.029**	0.237	0.110	**0.032**
GPR61	1	rs7550711	EUR, ALL	−348 505	0.001	0.001	1	rs11102023	SLS, ALL	A/C	0.989	0.635	0.248	**0.010**	0.640	0.248	**0.010**
SEC16B	1	rs543874	EUR	−58 402	0.122	–	–	rs200787218	SLS, ALL	A/C	0.328	0.198	0.051	***1.1E-04***	0.155	0.054	**0.004**
ADCY3	2	rs11676272	EUR	467 182	0.037	–	–	rs138716876	SLS, ALL	T/G	0.817	0.178	0.059	**0.003**	0.157	0.061	**0.010**
GNPDA2	4	rs13130484	EUR, ALL	384 441	0.004	0.029	1	rs75426894	SLS, ALL	T/C	0.976	0.388	0.153	**0.012**	0.374	0.153	**0.015**
TFAP2B	6	rs987237	EUR, ALL	−398 207	0.051	0.001	0.079	rs2709670	SLS, ALL	T/G	0.223	0.218	0.055	***9.4E-05***	0.245	0.056	***1.5E-05***
FAIM2	12	rs7132908	EUR, ALL	−27 580	0.004	0.022	0.298	rs297935	SLS, ALL	G/A	0.789	0.174	0.056	**0.002**	0.172	0.056	**0.003**
OLFM4	13	rs12429545	EUR, ALL	28 422	0.160	0.131	1	rs1072900	SLS, ALL	C/A	0.498	0.180	0.046	***1.1E-04***	0.169	0.051	***9.8E-04***
FTO	16	rs1421085	EUR, ALL	3771	0.002	0.052	1	rs62048379	SLS, ALL	A/C	0.060	0.314	0.095	***9.9E-04***	0.306	0.095	***1.3E-03***
MC4R	18	rs6567160	EUR, ALL	339 892	0.003	0.042	0.278	rs62092638	SLS, ALL	C/T	0.173	0.184	0.062	**0.003**	0.186	0.062	**0.003**
TMEM18	2	rs4854349/ rs4854348*	EUR, ALL	−203 384	0.007	0.001	0.072	rs11682609	SLS, ALL	A/C	0.959	0.323	0.128	**0.012**	0.314	0.128	**0.014**
ELP3	8	rs13253111/ rs7821358*	EUR, ALL	175 550	0.014	0.049	0.268	rs10097488	SLS, ALL	C/T	0.741	0.154	0.054	**0.005**	0.150	0.055	**0.006**
LMX1B	9	rs3829849/ rs62578126*	EUR, ALL	−72 782	0.009	0.024	0.329	rs10987410	SLS, ALL	C/T	0.068	0.267	0.095	**0.005**	0.258	0.095	**0.007**
RAB27B	18	rs8092503/ rs8085272*	EUR, ALL	−276 712	0.001	0.005	0.115	rs12961347	SLS, ALL	T/C	0.535	0.159	0.046	***4.9E-04***	0.160	0.046	***4.9E-04***
ADAM23	2	rs13387838/ No proxy		217 117	–	–	–	rs77068085	SLS, ALL	T/C	0.957	0.331	0.115	**0.004**	–	–	–

*Linkage disequilibrium (LD) (R^2) between tag and proxy SNPs ranged from 0.94 to 1.0.
[#]Conditional analyses results from PLINK, the beta coefficients for the population-specific tag SNPs still remained significant after entering the model with covariates pc1 to pc5 and corresponding EUR tag SNP for each locus. These results suggest that population-specific tag SNPs indeed have an effect independent of EUR tag SNP and other covariates.
β, beta coefficient; CEU, LD between SNPs in European descent population; Chile, LD between SNPs within SLS cohort; CHR, chromosome; D', D prime; EA, effect allele; EAF, effect allele frequency; OA, other allele; R2, LD for SNPs; SE, standard error; SNP, single nucleotide polymorphism.
Bold represents all nominally significant associations ($P < 0.05$); italics represents associations that meet significance for Bonferroni-correction ($P < 0.0017$).

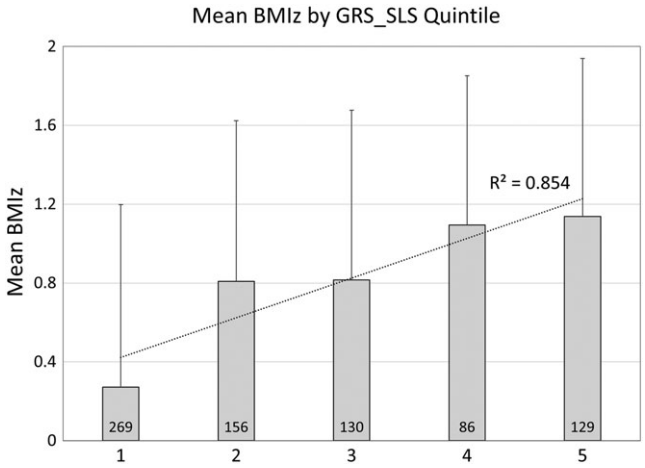

Figure 1 Plot of mean (+SD) BMIz across quintiles of GRS_SLS, with the dashed line highlighting the predicted linear fit and correlation coefficient.

identified similar patterns of association. Nominally significant association of BMIz with *FTO* genetic variants was first detected at age group 10, which is consistent (at or after age 7) with other studies (29). We observe a significant trend in the increase of the effect from age 5 to age 16 in line with findings in both EUR descent and Asian descent populations. Studies in EUR populations have shown an increase in the effect of rs9939609 across childhood and adolescence (30) and cumulatively support a peak in effect at age 20 (25). Similarly, the same variant exhibited higher magnitude of association with BMI and obesity in Chinese girls aged 12 to 18 years compared with 6 to 11 years (26). Our findings provide evidence for this pattern in Hispanic/Latinos; however, additional follow-up is needed to determine if the effects of the *FTO* locus also reach an apex in early adulthood. More importantly, the mechanism of timing of genetic effects at *FTO* and other loci may be useful for informing studies of primary and secondary obesity prevention across the life course.

Variants within the *OLFM4*, *ADCY3* and *FAIM2* loci are nominally associated with BMIz in SLS, but only at age 10 (our largest sample size). For these loci, effect estimates are larger at age 10 compared with effect estimates for BMIz measured at age 5 or 16. However, one of the limitations of the current study is the smaller sample sizes at ages 5 and 16, which prevented formal tests of heterogeneity between ages. Additionally, as the trend test only accounts for differences in effect estimates across age and therefore does not consider the correlation between measurements within individuals across time. Also, while our participants were measured at early childhood (adiposity rebound period), pre-puberty and adolescence, it is possible that hormonal changes occurring between 10- and 16-year visits and variation in the timing of puberty both within and across sexes have added heterogeneity to our results. Nonetheless, other studies using cross-sectional data have found similar patterns in changing allelic effect sizes across age. For example, in a study by Warrington *et al*. (28), the same BMI-associated significant genetic variants examined herein for *ADCY3* (rs11676272) and *OLFM4* (rs12429545) were associated with BMI at age 8 in a meta-analysis of two studies of EUR children, and the effect alleles illustrated a changing pattern of effect. While Warrington *et al*. observed an association between *OLFM4* variant and change in BMI, we did not observe any significant association with change in BMIz. Also, a similar to the pattern of association across age in SLS, these two variants displayed a pattern of greater genetic effects on weight at age 10 compared with ages 5 and 16 in EUR children (28).

In this cohort, BMIz is significantly associated with the EUR tag SNP, rs543874, in *SEC16B* at ages 10 after multiple test correction and nominally associated with BMIz at age 16, indicating an important role for this locus in Hispanic/Latino children. Nearby variants within this locus have previously exhibited significant age interaction effects in adult cross-sectional studies (10) contrasting effects in younger adults (≤50 years) to older adults (>50 years) and exhibiting larger effects in young adults. Similarly, in a separate study, the *SEC16B* locus displayed larger genetic effects on BMI in adolescents and young adults compared with middle aged (31). Also, this variant has also been significantly associated with change in BMI across adolescence and young adulthood in EUR and nominally associated in Hispanic/Latino children living in the USA (11). To

our knowledge, our study is the first to leverage longitudinal data to examine the differences in effects estimates of the *SEC16B* locus on BMIz across childhood and adolescence. Our study not only supports the relevance for this locus in Hispanic/Latinos but also underscores the importance of further investigations into the effects of this locus across the life course. Further analyses are needed to determine at what age the *SEC16B* has the greatest effect.

At each of the 14 loci with a EUR tag SNP available, we identified a more significant BMIz-associated SNP, 12 of which were not attenuated following conditional analyses indicating independence from the EUR tag SNP. Thus, in the SLS cohort, we not only replicated (9,28) known childhood obesity risk variants but also identified potential population-specific alternate genetic variants in Chilean children. These variants should be followed up in subsequent analyses of obesity-related phenotypes in ethnically diverse study populations, as they may provide new insights into biology underlying childhood obesity. For the locus near *TNNI3K*, we identified rs61790698 as the most significant SNP, which is in a nearby gene, crystalline zeta (*CRYZ*). A variant near *CRYZ*, rs3931020 (distance = 57.7 kb, R^2 = 0.016, D' = 1 with rs61790698 in CEU), has been associated with circulating resistin levels and increased risk of coronary heart disease in EUR adult GWAS study (32). Resistin levels are influenced by obesity and along the pathway between obesity and downstream cardiometabolic consequences (e.g. insulin resistance) (33), making *CRYZ* an interesting biological candidate for further study. Similarly, for rs13387838 near *ADAM23*, we could not identify a proxy SNP, but the most significant SNP within 1-MB interval was rs77068085, which is intronic to G protein-coupled receptor1 (*GPR1*). *GPR1* association with BMIz has not been previously reported and may represent better biological candidate as it was shown to regulate glucose homeostasis in obese mice (34).

For Hispanic/Latinos to fully benefit from precision medicine, we need to better characterize the genetic diversity of Hispanic/Latinos and determine how this diversity may influence unique underpinnings to disease (35). We addressed this issue by constructing an ancestry-specific GRS in a Chilean cohort with extensive admixture. The estimation of GRS–BMIz associations using published tag SNPs explained 1.94% of the variation in BMIz in our SLS cohort, close to the per cent variance explained reported in Felix *et al*. (2.0%). The small difference between these two estimates may be the result of differences in genetic and environmental influences over BMIz, the absence of rs13387838 in our SLS cohort and our focus on unweighted GRS. However, we also note that the GRS_EUR performed poorly compared with using ancestry appropriate tag SNPs identified in our Chilean population (13.63% variation compared with only 1.94%). While this result is promising, these results should be interpreted with caution, as winner's curse may play a role in the large estimate of variance explained. Regardless, our study adds to the recent body of literature that demonstrates the importance of GRS in assessing obesity risk (14,15) and the importance of selecting ancestry appropriate tag SNPs in future studies of disease prediction and clinical evaluation as it is shown that magnitude of association of GRS with BMI varies across different ethnic birth cohorts (13).

In summary, our study findings demonstrate an important role of genetic variants previously identified in EUR children in our Latin American paediatric population. Yet, we also demonstrate the importance of considering ancestry-specific variants for the most complete understanding of the role of genetic variation on risk of obesity during childhood. We observe distinct genetic effects across childhood and adolescence for *FTO*. Such patterning across the life course may be informative for understanding disease pathogenesis and in particular in children. From a public health standpoint, such findings are critical as once obesity is established in childhood, it is very difficult to reverse (36). Findings from this study yielded information on ancestry-specific alleles, biological mechanisms and candidates for prevention efforts with population-specific genetic risk estimates that are applicable in a variety of contexts, especially in ancestrally diverse populations.

Conflict of interest statement

The authors have no conflicts to disclose.

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A. E. J. and K. E. N. conceived of the study design; E. B. assisted in data collection; A. E. J., G. C., M. G. and Y. W. carried out data cleaning, analysis and generation of figures; A. E. J., G. C., M. G. and K. E. N. were involved in data interpretation. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Supporting information

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

Table S1. Descriptive characteristics of the Santiago Longitudinal Cohort Study participants. BMI – body mass index; BMIz – BMI Z-scores based on CDC reference

Table S2. Multiple linear regression results of BMIz with GRS' and other covariates. R2 – coefficient of determination; β – beta coefficient; SE – standard error, P – P-value.

Figure S1. Scatterplots comparing BMIz calculated using CDC reference panel to those from A. UK (used in Felix et al.) and, B. WHO reference panels.

Figure S2. Forest plot of each nominally significant locus ($P<0.05$ at ages 5, 10, and 16); estimated effect on BMI Z-score per effect allele by age. *Significant positive trend ($P<0.0001$).

Figure S3. Mean BMI Z-score plotted by genotype at the SEC16B and FTO loci at ages 5, 10, and 16. Dashed lines highlight the predicted additive effect of each allele on mean BMIz.