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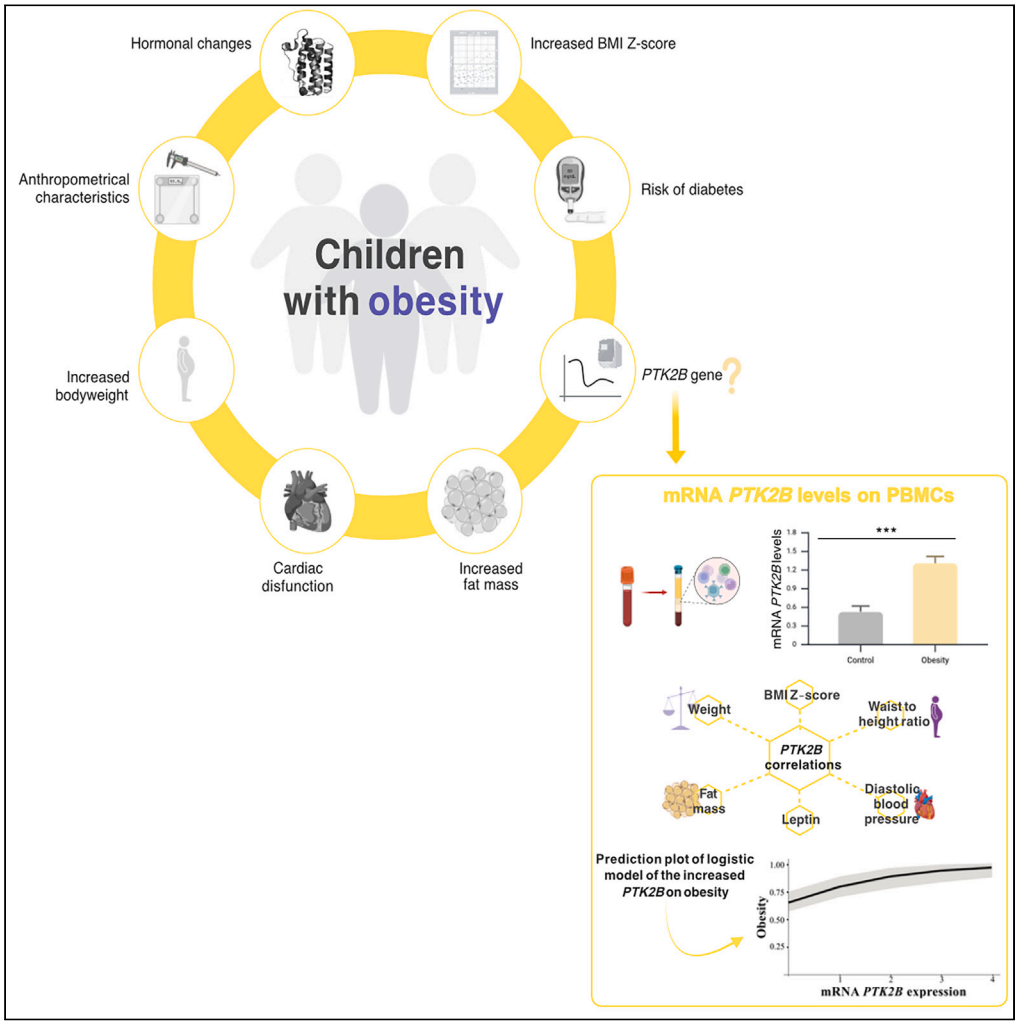

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Highlights

High levels of PTK2B gene expression might be a predictor of obesity development

Children and adolescents with obesity showed increased ARNm levels of *PTK2B* gene

PTK2B gene correlates positively with adiposity and leptin levels

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The PTK2B gene is associated with obesity, adiposity, and leptin levels in children and adolescents

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SUMMARY

Previous studies determined that Pyk2 is involved in several diseases in which the symptomatology presents alterations in energy balance. However, its role in obesity is poorly understood. To evaluate the metabolic role of the Pyk2 gene (*PTK2B*) in children and adolescents with obesity we measured its mRNA expression levels in peripheral blood mononuclear cells. For that we performed a cross-sectional study involving 130 Caucasian subjects that was divided into two groups according to BMI. Data showed increased *PTK2B* mRNA expression in children and adolescents with obesity. Interestingly, a positive correlation has been found between the levels of *PTK2B* with weight, BMI, BMI *Z* score, fat mass, waist circumference, waist to height ratio, diastolic blood pressure, and leptin. In addition, it is indicated that high levels of *PTK2B* gene expression might be a predictor of obesity development. This work provides important insights into the previously undescribed role of Pyk2 in obesity.

INTRODUCTION

The incidence of worldwide obesity has been increasing at an alarming rate in the last few decades in both adults and in children.[1,2] Childhood obesity is a determining risk factor for the development of obesity in adulthood. Thus, if obesity levels continue to increase, especially at younger ages, their effect on health and longevity in the coming decades could be very adverse.[3] In fact, obesity has been shown to have a substantial effect on longevity, the average lifespan of individuals with severe obesity being reduced by between 5 and 20 years.[4] Because early life determinants are a major factor in the rapid increase in obesity, the identification of new therapies that may aid in the prevention or amelioration of food intake disorders in children and adolescents is of vital importance.[1,2]

In this sense, peripheral blood mononuclear cells (PBMCs) provide an efficient source of early transcriptome indicators for identifying elevated risk in seemingly healthy adults.[5] PBMCs also provide a helpful and less invasive source of biomarkers.[6,7] PBMCs express a considerable proportion of the genes encoded by the human genome and function as "sentinel" cells capable of reflecting internal gene expression patterns. PBMCs can reflect gene expression signatures that are characteristic of different diseases such as metabolic syndrome or obesity.[8,9] Furthermore, it has been shown that PBMCs can modify their gene expression profile in response to hormonal or nutritional signals and

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Table 1. Summary of the anthropometric and biochemical characteristics of the participants in the cross-sectional study

	Lean (*n* = 48)	Obesity (*n* = 82)	*p*	TEST
Sexual development (prepuberty:puberty)	28:20	35:47	0.0849	P
Sex (girl:boy)	27:21	41:41	0.4911	P
Age (years)	10.3 (3.4)	11.2 (2.9)	0.1298	W
Tanner scale	1.9 (1.3)	2.3 (1.5)	0.0603	W
Weight (kg)	37.1 (13.9)	64.2 (22.0)	<0.0001	W
Height (cm)	139.9 (20.6)	149.1 (16.5)	0.0194	W
BMI	18.2 (2.9)	27.9 (4.9)	<0.0001	W
BMI *Z* score	0.2 (0.9)	3.5 (1.5)	<0.0001	W
Waist circumference (cm)	65.0 (12.1)	91.7 (13.8)	<0.0001	T
Waist to height ratio	0.5 (0.05)	0.6 (0.1)	<0.0001	W
Glucose (mg/dL)	78.7 (7.2)	79.4 (7.4)	0.6349	T
Insulin (mUI/L)	6.4 (3.9)	15.1 (9.0)	<0.0001	W
HOMA index	1.2 (0.7)	3.0 (1.9)	<0.0001	W
PTK2B gene expression	0.5 (0.6)	1.3 (1.0)	<0.0001	W
IGF-1 (ng/mL)	290.3 (187.4)	337.8 (168.3)	0.2009	W
Triglycerides (mg/dL)	53.4 (31.3)	74.9 (38.8)	<0.0001	W
Free fatty acids (mg/dL)	11.9 (5.9)	12.0 (5.1)	0.9240	T
Total cholesterol (mg/dL)	163.9 (31.8)	160.2 (33.3)	0.5428	W
LDL (mg/dL)	97.9 (33.6)	94.1 (32.5)	0.4510	W
HDL (mg/dL)	58.2 (14.9)	46.6 (12.5)	<0.0001	W
Systolic blood pressure	103.7 (9.2)	115.5 (12.2)	<0.0001	T
Diastolic blood pressure	59.0 (7.1)	68.8 (10.6)	<0.0001	We
Heart rate	85.4 (15.9)	84.6 (15.2)	0.8716	W
Leptin (ng)	4.5 (6.4)	13.0 (10.0)	<0.0001	W
FSH (mUI/L)	2.9 (2.5)	3.2 (2.3)	0.4617	W
TSH (mUI/L)	2.4 (1.2)	2.8 (1.2)	0.0588	W
fT3 (mUI/L)	4.4 (0.5)	4.2 (0.4)	0.3015	W
fT4 (mUI/L)	1.2 (0.1)	1.2 (0.1)	0.0922	W
Estradiol (pg/mL)	24.3 (28.8)	31.5 (44.5)	0.1139	W
Testosterone (ng/mL)	0.8 (1.4)	0.6 (0.7)	0.3933	W
Progesterone (ng/mL)	0.8 (0.9)	0.9 (0.7)	0.1952	W
Luteinizing hormone (UI/L)	1.8 (2.5)	2.3 (3.0)	0.4688	W
Vitamin - D (ng/mL)	19.5 (6.8)	17.9 (6.4)	0.1948	T
Total muscle mass (g)	2.5e+04 (6877.2)	3.2e+04 (1.1e+04)	0.0003	We
Total fat mass (g)	9620.1 (6526.8)	2.7e+04 (1.2e+04)	<0.0001	W
Fat mass (%)	23.9 (10.0)	43.6 (5.4)	<0.0001	We
Trunk muscle mass (g)	1.1e+04 (3138.0)	1.5e+04 (5351.1)	0.0029	W
Trunk fat mass (g)	4259.2 (3154.5)	1.4e+04 (6220.2)	<0.0001	W
Waist muscle mass (g)	1544.5 (456.4)	2061.7 (809.3)	0.0012	W
Waist fat mass (g)	713.9 (529.7)	2426.3 (1162.9)	<0.0001	W
Pelvis muscle mass (g)	3282.2 (1148.4)	4484.7 (1731.7)	0.0010	W
Pelvis fat mass (g)	1961.9 (1263.1)	4864.1 (2082.5)	<0.0001	W
Arm muscle mass (g)	2325.7 (693.5)	3319.5 (1240.3)	0.0001	W
Arm fat mass (g)	663.4 (497.4)	2393.5 (1266.9)	<0.0001	W

(*Continued on next page*)

Table 1. *Continued*

	Lean (n = 48)	Obesity (n = 82)	p	TEST
Leg muscle mass (g)	8402.0 (3251.9)	1.1e+04 (3854.7)	0.0007	W
Leg fat mass (g)	4260.9 (2897.0)	1.1e+04 (4901.2)	<0.0001	W

Note. Values are presented as mean (SD). Differences between groups were analyzed by Pearson's Chi-squared test (P), Wilcoxon rank-sum test (W), t-test (T), or Welch Two Sample t-test (We). Bold values mean significant statistical differences: Weight, Body Mass Index, Waist circumference, waist to height ratio, Fat mass, Insulin, HOMA index, TG, HDL–cholesterol, systolic blood pressure, diastolic blood pressure, Leptin, trunk fat and lean mass, waist fat and lean mass, pelvis fat and lean mass, arm fat and lean mass and leg fat and lean mass. HDL: high-density lipoprotein; HOMA: homeostatic model assessment; TG: triglycerides.

indeed mirror the expression profiles of metabolically active tissues that, on other hand, are more difficult to obtain in human biopsies, e.g., muscle, liver or adipose tissue.[10–14]

For example, PBMCs express leptin, visfatin, or ghrelin and their expression profile vary in response to other hormones, for example, insulin, glucagon, and leptin[10,15,16]; all of which are key hormones on energy balance regulation.[16–18]

The protein proline-rich tyrosine kinase 2 (Pyk2) is a protein also known to be related to adhesion focal tyrosine kinase, cell adhesion kinase b, and calcium-dependent tyrosine kinase.[19,20] Pyk2 is associated with poor prognosis and shortened survival in several types of cancers.[19,21,22] Previous studies determined that Pyk2 is also involved in some degenerative diseases and its gene, *PTK2B*, is a risk locus for Alzheimer's disease.[23,24] In addition, other results link the action of tyrosine kinase with resilience to anxiety and stress.[25] Interestingly all these diseases have a symptomatology that involves alterations in energy balance.[26–31] For example, important changes in appetite and body weight in these diseases are well described, however, the exact contribution of Pyk2 to these processes is still not well documented.

Furthermore, other reports found a link between Pyk2 and pathologies whose incidence has alarmingly surged due to obesity-related metabolic factors or low grade inflammatory states, such as cardiovascular diseases,[32,33] gut inflammation,[33,34] and metabolic associated liver diseases (MAFLD)[35] but it is also related to sepsis.[33] However, in spite of these interesting results, there is currently a great paucity of studies into the role of Pyk2 in obesity. In this respect, it was shown more than 15 years ago that a lack of Pyk2 in mice exacerbates weight gain as well as the development of high fat diet (HFD) induced glucose intolerance/insulin resistance, suggesting that Pyk2 may play a role in the development of obesity, insulin resistance, and/or diabetes.[36]

The main objective of this study was therefore to evaluate whether or not obesity influences the mRNA expression of *PTK2B* in PBMCs in children and adolescents. In addition, it aimed to identify the correlations between PBMC-derived *PTK2B* and clinical obesity indices. As secondary objectives, we aimed to determine whether PBMCs *PTK2B* mRNA expression correlates with different anthropometric and biochemical parameters.

RESULTS

PTK2B gene expression is upregulated in children and adolescents with obesity

Obesity causes the expected increase in weight, body mass index (BMI), BMI *Z* score, waist circumference, waist to height ratio, fat mass, HOMA index, insulin levels, triglycerides (TG), low-density lipoprotein (LDL), systolic and diastolic blood pressure, free thyroxine (fT4), and leptin compared to control group (Table 1). Conversely, other circulating factors that are considered beneficial against the disease such as high density lipoproptein (HDL) are significantly decreased in children with obesity compared to children without (Table 1).

PTK2B is elevated in some inflammatory diseases[33,34] and obesity is a chronic low-grade inflammatory disease.[37,38] Moreover, it has been found that obesity induces an upregulation of the Pyk2 levels in peripheral tissues in mice.[36] As a result, we speculate that the levels of this tyrosine kinase might be increased in human obesity. Considering the difficulty in measuring Pyk2 levels directly from plasma and the wide use of PBMCs as a reliable surrogate marker of tissue expression, we measured *PTK2B* gene levels from PBMCs across our cohort. Our results show that *PTK2B* expression is significantly elevated in children with obesity compared to the lean controls (Figure 1A).

The dataset was further scrutinized, with analyses conducted separately in girls and boys. In both groups, *PTK2B* expression showed a notable increase in girls and boys presenting obesity (Figure 1A). Therefore, this data indicate that the upregulation of *PTK2B* gene in children with obesity is independent of gender.

To assess the potential impact of the sexual development stage on *PTK2B* expression, we conducted an analysis based on the pubertal stage. Regardless of whether presenting obesity or normal weight, both children (pre-pubertal stage) and adolescents (pubertal stage) exhibited comparable gene expression patterns (Figure 1B).

Finally, to explore potential gender-specific variations in our cohort independent of the state of obesity, we compared the levels of *PTK2B* between girls and boys in the total population (with and without obesity). On completing this comparison, we were unable to find any sexual dimorphism in *PTK2B* expression. In addition, when we divided the population according to both the stage of puberal maturation and by sex, we found that the *PTK2B* gene expression in both sexes is also similar in different stages of sexual development (Figure 1C).

PTK2B gene correlates with adiposity and leptin levels in children and adolescents

Once we found that *PTK2B* expression is upregulated in obesity, the next step was to correlate these levels with the anthropometric and biochemical factors. The correlation study was performed by avoiding categorical variables. Our results showed a significant positive

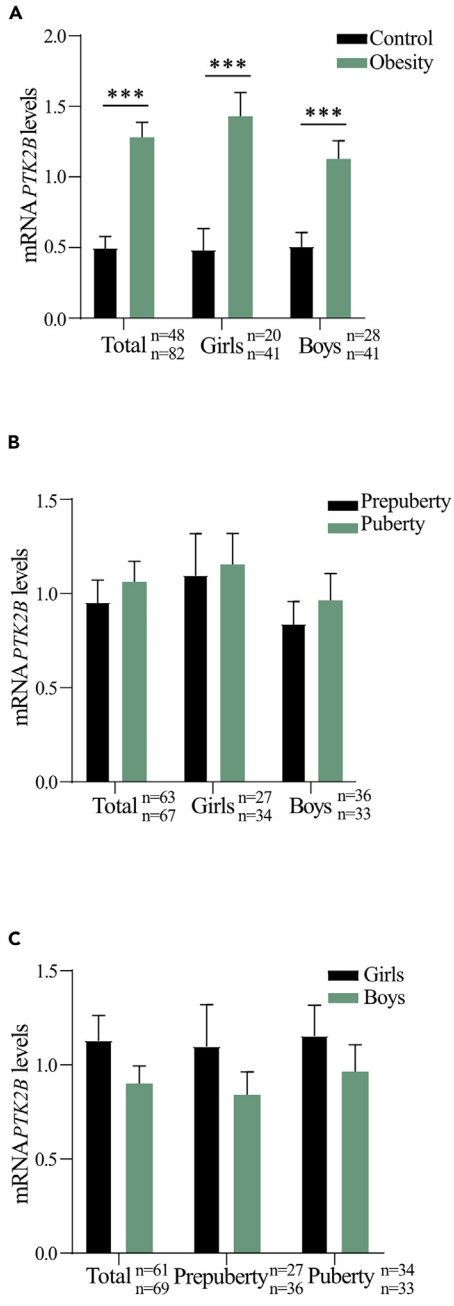

Figure 1. *PTK2B* gene expression is upregulated in children and adolescents with obesity

(A) *PTK2B* expression in children with obesity. *PTK2B* mRNA levels in children with normal weight and children with obesity of both sexes. *PTK2B* mRNA levels in girls with normal weight and girls with obesity. *PTK2B* mRNA levels in boys with normal weight and boys with obesity.

(B) *PTK2B* expression in the study population based on the pubertal stage. *PTK2B* mRNA levels in prepubescent and pubescent individuals of both sexes. *PTK2B* mRNA levels in prepubescent and pubescent girls. *PTK2B* mRNA levels in prepubescent and pubescent boys.

(C) *PTK2B* expression in the study population according to gender. *PTK2B* mRNA levels in prepubescent girls and boys. *PTK2B* mRNA levels in pubescent girls and boys Differences between groups were analyzed by t test. Data are expressed as mean ± SEM. *** p value <0.001.

correlation between *PTK2B* levels and weight (Table 2; Figure 2A), BMI (Table 2; Figure 2B), waist circumference (Table 2; Figure 2C), fat mass (Table 2; Figures 2D and 2E) diastolic blood pressure (Table 2; Figure 2F), and leptin levels (Table 2; Figure 2G).

Current evidence shows biological and genetic differences in adipose tissue depending on its anatomical location. In particular, visceral fat distribution in obesity is closely linked to metabolic complications.[39] In this regard and taking advantage of the measurement of body composition with DEXA we correlated the levels of PTK2B with the fat mass at different body locations. We found a positive correlation between our

Table 2. Correlations made by Pearson Correlation Coefficient

	Pearson R^2	*p*
Age (years)	−0.048	0.585
Weight (kg)	**0,184**	**0,035**
Height (cm)	−0.038	0.666
BMI	**0,3309**	**0,0001**
BMI *Z* score	**0,416**	**<0,0001**
Waist circumference (cm)	**0,254**	**0,003**
Waist to height ratio	**0,38**	**<0,0001**
Glucose (mg/dL)	0.028	0.747
Insulin (mUI/L)	0.071	0.416
HOMA index	0.063	0.473
IGF-1 (ng/mL)	−0.073	0.407
Triglycerides (mg/dL)	−0.057	0.517
Free fatty acids (mg/dL)	0.078	0.374
Total cholesterol (mg/dL)	−0.034	0.694
LDL (mg/dL)	0.065	0,4584
HDL (mg/dL)	−0.082	0.348
Systolic blood pressure	0.146	0.096
Diastolic blood pressure	**0,175**	**0,046**
Heart rate	−0,02	0.814
Leptin (ng)	**0,173**	**0,048**
FSH (mUI/L)	0.035	0.691
TSH (mUI/L)	−0.046	0.596
fT3 (mUI/L)	0,11	0.209
fT4 (mUI/L)	−0.014	0.866
Estradiol (pg/mL)	0.031	0.728
Testosterone (ng/mL)	−0.004	0.961
Progesterone (ng/mL)	0.048	0.583
Luteinizing hormone (UI/L)	−0.013	0.879
Vitamin - D (ng/mL)	−0,0187	0.832
Total muscle mass (g)	0,05	0.567
Total fat mass (g)	**0,214**	**0,014**
Fat mass (%)	**0,288**	**<0,0001**
Trunk muscle mass (g)	0.041	0.641
Trunk fat mass (g)	**0,199**	**0,022**
Waist muscle mass (g)	0.092	0.297
Waist fat mass (g)	**0,223**	**0,01**
Pelvis muscle mass (g)	0.102	0.243
Pelvis fat mass (g)	**0,196**	**0,025**
Arm muscle mass (g)	0.099	0.259
Arm fat mass (g)	**0,221**	**0,011**
Leg muscle mass (g)	0.084	0,34
Leg fat mass (g)	**0,176**	**0,045**

Note. Bold values mean significant statistical differences with a *p* value <0.05.

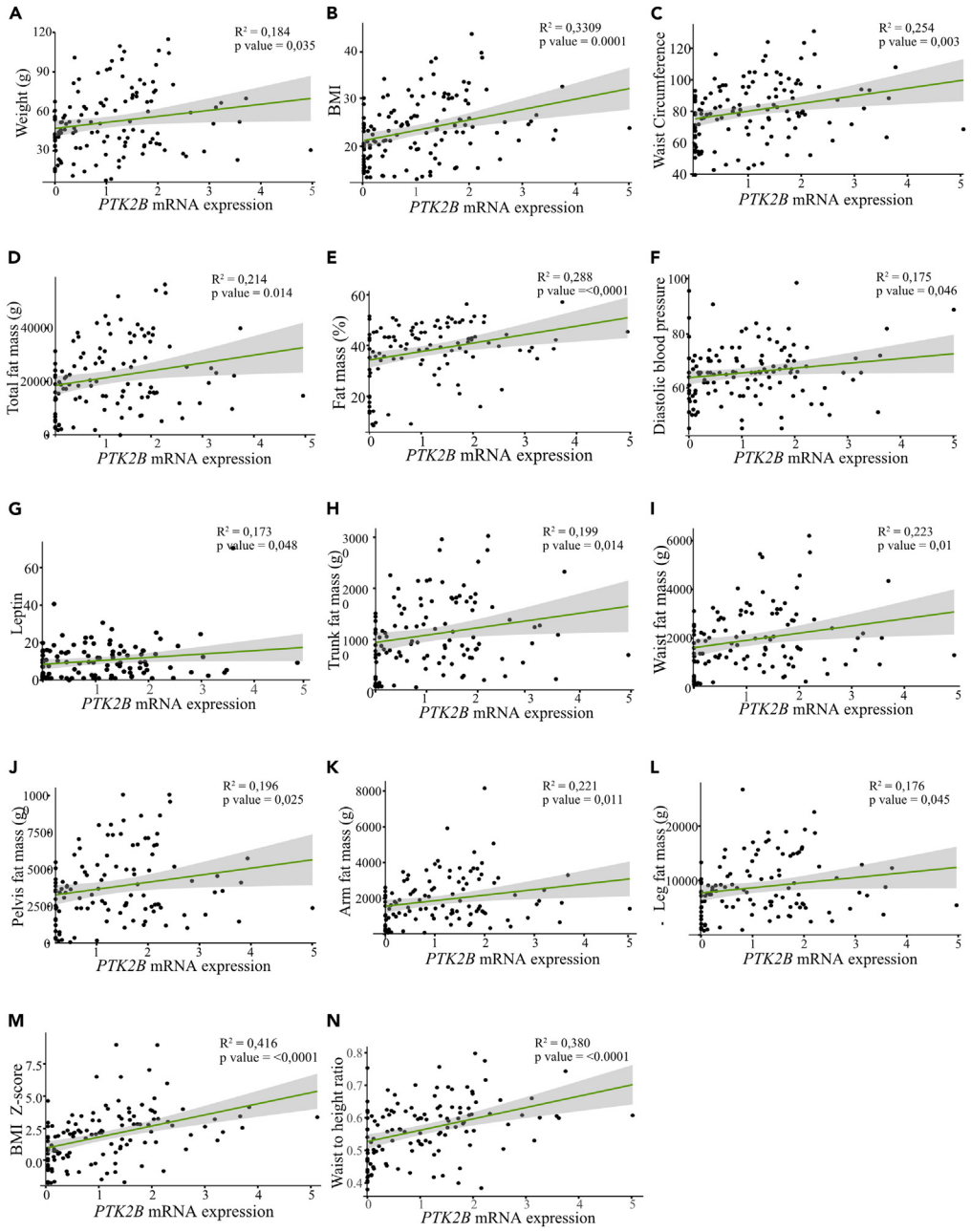

Figure 2. Correlation analysis of the study population

Bivariate correlation between *PTK2B* gene expression and weight (A), BMI (B), Waist circumference (C), total fat mass (D), fat mass % (E), diastolic blood pressure (F), Leptin (G), trunk fat mass (H), waist fat mass (I), pelvis fat mass (J), arm fat mass (K), leg fat mass (L), BMI *Z* Score(M) and waist to height ratio (N). BMI: Body mass index. Circles represent the plotted values obtained for each variable while the line represents the best fit for the correlation between them ± Confidence interval 95%.

gene expression levels with fat mass from the trunk (Table 2; Figure 2H), waist (Table 2; Figure 2I), pelvis (Table 2; Figure 2J), arms (Table 2; Figure 2K), and legs (Table 2; Figure 2L). We were interested in ascertaining the obesity progression in different age categories and to do so we found that the distribution of fat mass during this early life course is similar between fat depots with minimum levels at 9 years old and maximum levels at 14 years old (Figure S1).

In order to avoid confusing factors in our analyses, we also correlated the levels of *PTK2B* with age and with BMI *Z* score that is a relative measure of body mass index taking age into account. To account for this we split our population, separating boys and girls, into two age categories, 3.5–9.3 years old and 9.4–15.3 years old. We did not find a correlation with this measurement (see Table S1). Interestingly, when we use the BMI *Z* score in our analysis we found a positive correlation of *PTK2B* and this index in children and adolescents with obesity

Table 3. Selection of variables in obesity

	OR (CI 95%)	*p*
Sexual development (prepuberty-puberty)	1.88 (0.92–3.91)	0.086
Sex (girl-boy)	1.29 (0.63–2.65)	0.491
Age (years)	**1.33 (0.93–1.91)**	**0.116**
Tanner scale	**1.40 (0.97–2.10)**	**0.084**
Weight (kg)	7.17 (3.69–16.13)	<0.001
Height (cm)	1.64 (1.14–2.41)	0.009
BMI	870.06 (81.23–28788.72)	<0.001
BMI *Z* score	2050.6 (143.31–103480.19)	<0.001
Waist circumference (cm)	16.79 (6.89–53.35)	<0.001
Waist to height ratio	59.97 (16.50–344.14)	<0.001
Glucose (mg/dL)	1.09 (0.76–1.57)	0.634
Insulin (mUI/L)	**3.45 (2.01–6.45)**	**<0.001**
HOMA index	**3.42 (1.96–6.49)**	**<0.001**
***PTK2B* gene expression**	**3.29 (1.97–5.94)**	**<0.001**
IGF-1 (ng/mL)	1.28 (0.89–1.89)	0.203
Triglycerides (mg/dL)	**2.12 (1.32–3.74)**	**0.005**
Free fatty acids (mg/dL)	1.02 (0.71–1.47)	0.921
Total cholesterol (mg/dL)	0.90 (0.63–1.28)	0.546
LDL (mg/dL)	**0.90 (0.63–1.29)**	**0.554**
HDL (mg/dL)	**0.44 (0.28–0.66)**	**<0.001**
Systolic blood pressure	**2.84 (1.78–4.86)**	**<0.001**
Diastolic blood pressure	**2.72 (1.75–4.52)**	**<0.001**
Heart rate	0.96 (0.67–1.37)	0.802
Leptin (ng)	**3.50 (1.95–6.76)**	**<0.001**
FSH (mUI/L)	1.11 (0.78–1.62)	0.560
TSH (mUI/L)	**1.40 (0.96–2.11)**	**0.091**
fT3 (mUI/L)	0.83 (0.57–1.19)	0.305
fT4 (mUI/L)	**0.69 (0.46–0.99)**	**0.054**
Estradiol (pg/mL)	1.18 (0.81–1.98)	0.440
Testosterone (ng/mL)	0.85 (0.58–1.21)	0.349
Progesterone (ng/mL)	1.07 (0.75–1.58)	0.713
Luteinizing hormone (UI/L)	1.16 (0.81–1.73)	0.444
Vitamin - D (ng/mL)	0.79 (0.55–1.13)	0.203
Total muscle mass (g)	1.77 (1.19–2.77)	0.008
Total fat mass (g)	5.20 (2.87–10.66)	<0.001
Fat mass (%)	66.67 (14.18–577.12)	<0.001
Trunk muscle mass (g)	1.82 (1.21–2.90)	0.007
Trunk fat mass (g)	6.12 (3.25–13.16)	<0.001
Waist muscle mass (g)	1.97 (1.28–3.22)	0.004
Waist fat mass (g)	7.28 (3.66–16.61)	<0.001
Pelvis muscle mass (g)	5.03 (2.77–10.31)	<0.001
Pelvis fat mass (g)	5.03 (2.77–10.31)	<0.001
Arm muscle mass (g)	2.29 (1.47–3.80)	0.001
Arm fat mass (g)	7.38 (3.66–17.14)	<0.001

(*Continued on next page*)

Table 3. *Continued*

	OR (CI 95%)	*p*
Leg muscle mass (g)	1.88 (1.26–2.91)	0.003
Leg fat mass (g)	4.43 (2.50–8.77)	<0.001

Note. Univariated logistic regressions. OR: the odds ratio. CI 95%: Confidence Interval 95%. *p* value. Bold values mean those variables with a significance level lower to 0.2. HDL: high-density lipoprotein; HOMA: homeostatic model assessment; TSH: thyroid stimulating hormone, fT4 (free thyroxine).

(Table 2; Figure 2M). Moreover, due to the fact that puberty could be further subdivided into early (Tanner index 2–3) and late stages (Tanner index 4–5) we wondered if the positive correlation of *PTK2B* levels with the biochemical and anthropometric factors pointed out before would be replicated in taking into account these pubertal stages separately. Our results show that in prepubertal children with obesity, the levels of *PTK2B* are correlated positively with BMI *Z* score and testosterone while both in the early and post pubertal stages our results are very similar to the data presented in Figure 2 (see Table S2; Figure S2). Of note is that the positive correlation between *PTK2B* and BMI *Z* score disappear if we made these analyses with the case and controls separately (see Table S3). This is probably due to the fact that when comparing the groups separately the number of individuals per group is reduced. Finally due to height also being one parameter that could interfere with our analysis we also correlated the levels of *PTK2B* with the waist to height index. We again found a positive correlation with this parameter (Table 2; Figure 2N).

These results fit well with our hypothesis that due to high weight, BMI, waist circumference, and high fat mass are the terms that define obesity and are positively correlated with *PTK2B* levels. Similarly, obesity is characterized by high diastolic blood pressure and a state of leptin resistance when the level of this hormone is high and here, we show that *PTK2B* is positively correlated with this cardiometabolic factor and/or with this endocrine factor. This is an interesting and significant finding that is well worth highlighting.

Obesity in children is associated with *PTK2B* gene expression, HDL, and systolic blood pressure

To elucidate which factors or variables can explain the occurrence of obesity among children and adolescents in our study population, a binary logistic regression was conducted, incorporating both biochemical and anthropometric parameters, as well as an assessment of the expression of *PTK2B* gene. We performed a selection of the variables by logistic regression using simple models comparing all variables with the presence of obesity. The selection criteria for this model included a variable when the significance level fell strictly below 0.2 based on simple logistic regression and criteria provided by the research (Table 3; *p* value).

Focusing on the results of the univariate logistic regressions, the variables with a *p* value higher than 0.2 were eliminated. The variables eliminated were sex, free fatty acids (FFA), total cholesterol, LDL, glucose, insulin growth factor 1 (IGF-1), heart rate, follicle stimulating hormone (FSH), free triiodothyronine (fT3), estradiol, testosterone, progesterone, luteinizing hormone (LH), and vitamin D (vit D). In addition, the variables highly related to obesity and constitutive of the meaning of the term were eliminated to avoid redundancy, and, in addition, to try to give prominence or statistical space to other factors. Therefore, the variables eliminated were weight, height, BMI *Z* score, waist circumference, waist to height ratio, fat mass, and muscle mass.

Next, we performed an adjusted logistic model as the optimal model that would best explain obesity in children based on the lowest AIC value (Table 4). This model includes *PTK2B* gene, HOMA index, HDL, systolic blood pressure, leptin, thyroid-stimulating hormone (TSH), and fT4 (Table 3).

Data were standardized by scaling the values of variables to achieve a mean of "0" and a standard deviation of "1". Variables with an odds ratio greater than 1 indicate a risk of obesity: conversely, variables with an odds ratio of less than 1 play a protective role against obesity. It is important to note that in this final adjusted model only the statistically significant factors are the ones that can act as predictors of the disease. Therefore, these variables are *PTK2B*, HDL cholesterol, and systolic blood pressure. For every 1-unit increase in the expression of the PTK2B

Table 4. Univariated and multivariate regression model of obesity status in the selected variables

	OR (CI 95%)	*p*	OR (CI 95%)	*p*
HOMA index	1.58 (0.19–12.83)	0.669	1.33 (0.83–2.13)	0.232
TSH (mUI/L)	1.32 (0.84–2.09)	0.231	1.36 (0.91–2.04)	0.135
fT4 (mUI/L)	0.71 (0.47–1.09)	0.116	0.71 (0.47–1.07)	0.099
Leptin (ng)	1.46 (0.92–2.30)	0.107	1.50 (0.97–2.32)	0.067
Systolic blood pressure	**1.50 (0.92–2.43)**	**0.103**	**1.58 (1.02–2.45)**	**0.039**
HDL (mg/dL)	**0.60 (0.38–0.94)**	**0.026**	**0.58 (0.38–0.89)**	**0.012**
***PTK2B* gene expression**	**2.12 (1.38–3.26)**	**0.001**	**2.09 (1.38–3.14)**	**<0.001**
AIC	–	–	118.5	–

Note: Bold values mean significant statistical differences. cOR: Crude Odds Ratio, univariated logistic regressions. cP value: *p* value of cOR. aOR: Adjusted Odds Ratio, multivariated logistic regressions. aP-value: *p* value of aOR. CI 95%: Confidence Interval 95%.

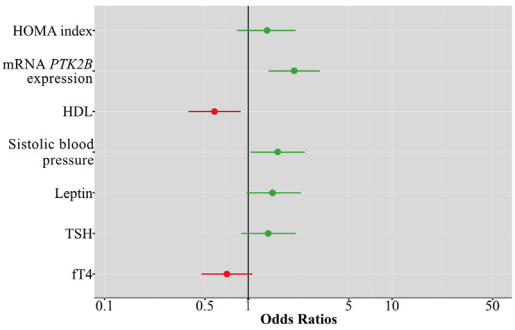

Figure 3. Odds Ratio Plot of adjusted binary logistic regression model

PTK2B gene, HOMA index, HDL, systolic blood pressure, leptin, TSH and fT4. HOMA: homeostatic model assessment, HDL: high-density lipoprotein; TSH: thyroid-stimulating hormone and fT4: free thyroxine. Odds ratio ± Confidence interval 95%.

gene, HOMA index, TSH, systolic blood pressure, or leptin there is an associated 2.09, 1.33, 1.36, 1.58, or 1.50 unit increase in the risk of obesity, respectively. Similarly, a 1-unit increase in HDL—cholesterol, corresponds to a protective factor of 0.58 units against obesity. In addition, a 1-unit increase in fT4 indicates a protective factor of 0.71 units against obesity (Table 4; Figure 3).

Finally, we compare the *PTK2B* expression with the degree of obesity and with this model's information, we predict that the increased mRNA levels of *PTK2B* makes an increased probability of developing obesity (Figure 4).

DISCUSSION

Pyk2 is a pleiotropic non-receptor tyrosine kinase recently identified as a potential therapeutic target for the treatment of some cancers,[19,21,22] neurodegenerative diseases,[23,24] cardiovascular diseases,[32] and MAFLD.[35] However, the potential role of Pyk2 in obesity has remained largely unexplored. In this work, we find that *PTK2B* gene expression is upregulated in children with obesity. Consistent with this finding is the fact that Pyk2 is considered an inflammatory marker[33,34] and that obesity is considered a chronic low-grade inflammatory disease.[37,38]

Is important to highlight that Pyk2 is a tyrosine kinase and its measurement directly from plasma is difficult to achieve. We therefore propose the use of PBMCs for several reasons: (a) the use of PBMCs provides a useful and minimally invasive source of biomarkers, (b) PBMCs express around 80% of the genes encoded by the human genome, and (c) PBMCs are able to reflect the expression profiles of internal tissues[6,9,40]

In fact, PBMC expression changes correlate with those of internal tissues such as those of the liver, adipose tissue, and the hypothalamus, after short term changes in nutritional status such as after fasting and refeeding.[12,14,41,42] Interestingly, the genes involved are those related to the lipid metabolism pathway.[43]

Specifically, overnight fasting decreases PBMC gene expression in genes involved in fatty acid synthesis such as sterol regulatory binding protein-1 (SREBP-1) and fatty acid synthetase.[12,14] Conversely, fasting increases PBMC expression of genes involved in fatty acid oxidation, such as carnitine palmitoyl transferase 1 (CPT1a).[12,41] A similar effect was found in the liver.[44]

Moreover, the expression profile of the sterol metabolism genes resembled that previously described for the liver, decreasing in response to fasting conditions and recovering the levels found in fed animals after 6 h of refeeding.[45]

Consistent with these results is that the long-term changes in nutritional status such as addition of HFD over several weeks upregulates the expression of CPT1a and reduces the expression of fatty acid synthase and Srebp1 in PBMC[46] and these changes are similar to the changes observed in liver and adipose tissue in this context.

Furthermore, the expression of some genes implicated in the thermogenesis or "browning" effect in PBMC follow the same regulatory behavior as that observed in the white and brown adipose tissue of animals fed with hyperlipidic diets.[47] In the same way, PBMC also reflect genetic changes in pathways related to thermogenic induction and "browning", as well as increased gene expression levels in genes related to β-oxidation pathways, in the white and brown adipose tissue depots of rats exposed to cold.[48]

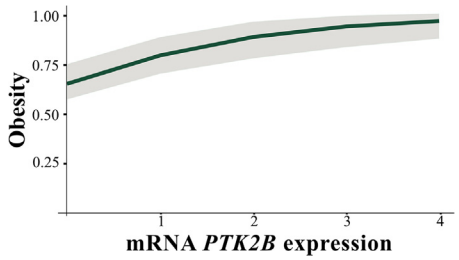

Figure 4. Predictions plot of obesity by changes in the expression of *PTK2B*

Regression line ± Confidence interval 95%.

Interestingly, a strong correlation was observed between transcript expression levels of PBMCs and a skeletal muscle biopsy after *n*-3 PUFA supplementation in subjects with obesity.[13]

With regard to this last issue, it is important to note that our results from mRNA of *PTK2B* in PBMCs are similar to those that show an upregulation of protein levels of Pyk2 in peripheral tissues such as liver and white adipose tissue in regard to in diet-induced obesity mice.[36]

Importantly, we show the positive correlation between *PTK2B* with BMI, waist circumference and fat mass. Genome-wide association studies (GWASs) revealed an association between an intronic single nucleotide polymorphism in *PTK2B* and BMI.[47] However, association with non-coding variants does not identify, which variant is causally related to the trait nor which gene is affected by the causal variant.[48] In fact, the associated SNP is closer to the transcriptional start site of other genes in the region, such as CHRNA2 or EPHX2, than to that of *PTK2B*. Open Targets Genetics[49,50] a resource that makes use of different functional genomics data to prioritize genes at each genome-wide significant locus, considers *PTK2B* as the most likely gene involved in association with BMI at this locus based on the largest GWAS to date[51] (https://genetics.opentargets.org/study-locus/GCST009004/8_27403621_C_G). However, the "locus-to-gene" score is far from conclusive, assigning a probability for *PTK2B* to be the causal gene at this locus of, at most, 44%. Thus, our results shed light on the interpretation of this association signal, adding data to increase the evidence of *PTK2B* as the causal BMI susceptibility gene in that region. Interestingly, intronic SNPs at PTK2B are also genome-wide significantly associated with hematological traits of relevance in inflammatory responses, such as leukocyte counts or eosinophil percentage of leukocytes.[52] This accords with our hypothesis that elevated levels of *PTK2B* could be a biomarker of the disease and implies that Pyk2 is positively correlated with relevant anthropometric factors associated with obesity. This is to be expected if we consider that obesity is directly proportional to the amount of fat and waist circumference and indeed is defined by high BMI.

Moreover, and in order to avoid any interference arising from factors such as age or height in our analysis we correlate the expression of our gene of interest with BMI *Z*score and the waist to height ratio. In considering our results, we conclude that the positive correlation between *PTK2B* levels and these obesity indices is independent of the height and age of the patients with obesity. Furthermore, *PTK2B* gene expression is correlated positively with the endocrine factor leptin. Leptin is a hormone secreted mainly by white adipose tissue and in proportion to the amount present, so it is therefore reasonable to think that leptin as a surrogated marker of fat mass and so is also associated with high *PTK2B* expression. Moreover, this observation suggests a possible mechanism by which Pyk2 could interfere in obesity. In fact, the relationships between these factors were previously reported in one study in mice where leptin treatment normalizes the hypothalamic levels of Pyk2 induced after fasting.[40]

After using different statistical models, we found that obesity in our children cohort is explained by *PTK2B* gene expression, HDL—cholesterol, and fT4.

It is well known that HDL produces an array of beneficial effects on the body. Decreased HDL, on the other hand, is strongly associated with obesity. Moreover, obesity is an important risk factor for a decrease in HDL levels. Indeed, low circulating HDL is also a marker of insulin-resistance, a pathological precondition of obesity.[53]

On the other hand, it has been shown that one of the main alterations reported in the thyroid axis profile of patients with obesity is a decrease in fT4 levels.[54] We therefore think it is reasonable to conclude that HDL and fT4 are protective factors against the development of obesity in our cohort.[55,56]

Significantly, our analysis shows that high *PTK2B* levels correlate significantly with obesity, which could be indicative of an elevated risk factor for obesity, which should be determined in future studies. Specifically, *PTK2B* is associated with a 2.09-unit increase in the risk of developing obesity, while with HOMA index, TSH, systolic blood pressure or leptin there is an associated 1.33, 1.36, 1.58, and 1.50-unit increase respectively in the risk of obesity. Opposite HDL and fT4 are associated with 0.58 and 0.71 units, respectively, for protection against the development of obesity.

Finally, we produced a prediction plot of obesity against changes in the expression of *PTK2B* and found that the higher the degree of obesity the higher the expression of *PTK2B*. These data suggest that the *PTK2B* gene in PBMCs might serve as a predictor for future obesity and metabolic disease development in early childhood.

In conclusion, we have found a link between Pyk2 gene expression, and the different factors associated with obesity such as weight, BMI, fat mass, waist circumference, systolic blood pressure, and leptin in humans. Moreover, the *PTK2B* gene could be used as a predictor of future development of obesity in children and adolescents and might provide early interventions to limit obesity progression. Therefore, our results highlight, the significant role of the *PTK2B* gene in pediatric obesity.

Due to Pyk2 being involved in the pathophysiology of different diseases associated with obesity such as some types of cancer, in addition to neurodegenerative, cardiovascular and inflammatory diseases and MAFLD, identifying the common molecular mechanisms underlying these disorders will help us to find more effective methods for their prevention with the accompanying benefit of extending healthy lifespans in humans. In this sense, our discoveries could have a therapeutic implication since Pyk2 could be a possible target to act on. Therefore, the development of a specific pharmacological antagonist for Pyk2 or a more precise system by which the expression of the *PTK2B* gene could be genetically inhibited (e.g., adenoassociated viruses, small extracellular vesicles, or the use of nanoparticles) in a safe and effective manner and without side effects could be an option. However, future studies will be necessary to clarify this issue.

Limitations of the study

Our main limitation is that our analysis implies one single gene. Is important to note that many other known and unknown genes are implicated in the development of obesity and Pyk2 is only one of the signals that are involved in individuals with obesity. More studies are therefore

needed to ascertain the many other pathways implicated in the pathophysiology of this disease. Moreover, further experiments are required to ascertain the contribution that *PTK2B* makes to the development of obesity.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be handled by the lead contact, Omar Al-Massadi (omar.al-massadi.iglesias@sergas.es).

Materials availability

This study did not generate new or unique reagents.

Data and code availability

- The patients' data reported in this study cannot be deposited in a public repository because they were used with permission for this study with restrictions that do not allow for the data to be redistributed or made publicly available.
- This paper does not report the original code.
- Any additional information required to respond to any question related to the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

E.P. investigation, formal analysis, writing original draft, visualization. R.P.-L., P.J.-F., D.M.-M., B.B-G., M. V., V.P.-L, R.V.-C., A.C.M., J.C.-A, J.C., A.E.-G., M.Q. investigation, formal analysis and writing review—editing. Visualization, R.L., L.-M.S., and O.A.-M. conceptualization; resources, and supervision; writing review—editing, visualization; project administration, and funding acquisition. All authors discussed the results, commented on the manuscript before submission, and agreed with the final submitted manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
PBMCs	Investigation in Nutrition, Growth and Human Development Unit of Galicia (Clinical University Hospital of Santiago de Compostela)	Central Ethics and Research Committee of the Galician Autonomous Community (2013/256)
Critical commercial assays		
E.Z.N.A.® Cycle Pure Kit (V-spin)	Omega Bio-tek	D6492-00S
One-step NZYSpeedy RT-qPCR Green kit, ROX plus	NZYtech	MB34402
LabAssay (TM) NEFA	FUJIFILM Wako Shibayagi Corporation	633–52001
PolymorphPrep solution	Progen	1895
TRIzol reagent	Thermo Fisher Scientific	15596018
Oligonucleotides		
β - actin Human Forward: CACAGAGCCTCGCCTTTGC	Thermo Fisher Scientific	N/A
β - actin Human Reverse: CCACCATCACGCCCTGG	Thermo Fisher Scientific	N/A
PTK2B Human Forward: TTAACCAATCCCCGGGAACC	Thermo Fisher Scientific	N/A
PTK2B Human Reverse: TTCAAACAAGCCCCGTTCCA	Thermo Fisher Scientific	N/A
Software and algorithms		
GraphPad Prism	GraphPad Software	https://www.graphpad.com/
R Studio	The R Foundation	https://posit.co/download/rstudio-desktop/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

A cohort of female and male patients was selected including a total of 130 Spanish Caucasian children (Tanner index 1) and adolescents (Tanner index 2-5) from 3 to 15 years old. Enrollment into the study occurred between June 2013 to present.[57–59] The patients were recruited by the Investigation in Nutrition, Growth and Human Development Unit of Galicia (Clinical University Hospital of Santiago de Compostela). This cohort was classified according to body mass index (BMI) as children with obesity (n = 82) or normal weight (n = 48) using the age and sex-specific BMI cut-off points of the International Obesity Task Force, equivalent to adult values of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity.[60] We tested the influence of gender and sexual development in our main variables and the analysis allow us to conclude that they are independent.

This is a cross-sectional study, and it adhered to the principles outlined in the Declaration of Helsinki and all procedures were approved by the Central Ethics and Research Committee of the Galician Autonomous Community (code 2013/256). The inclusion of all the children in the study was obtained with written, informed and signed parental consent. Individuals who had taken any medication or had any chronic disorder that could potentially influence the study outcomes were excluded from participation in the study. Finally, the manuscript does not present any original data on any species other than the ones originated from human samples.

Clinical examination and blood sampling

Anthropometric measurements were taken in the morning by a single paediatrician with participants wearing only underwear and no shoes. Body weight was recorded using a digital electronic balance (Seca mod. 813. gmbh & CO) with an accuracy of 0.1 kg, while height was measured with a calibrated wall-mounted stadiometer (Seca mod. 213. gmbh & CO) with an accuracy of 0.1 cm. BMI was calculated by dividing the weight in kilograms by the square of the height in meters. Waist circumference (WC) was measured in fasting state by applying an inelastic tape horizontally midway between the lowest rib margin and the iliac crest of the standing child at the end of a gentle expiration.

Whole-body measurement is performed to determine bone mass, non-bone fat-free mass, and fat mass. Using the General Electric LunarEncore® DEXA: X-ray source (38keV and 70keV photons).

Clinical examinations were conducted by trained paediatricians using standardized methods. Pubertal development was determined using Tanner's criteria, according to gender i.e. stage 1 is prepubertal and stages 2 to 5 pubertal, with 2-3 early and 4-5 late stage.

Blood samples were collected after a 12-hour overnight fasting period.

METHOD DETAILS

Biochemical assays

Plasma glucose, total cholesterol, and TG levels were assessed using the Advia 2400 Chemistry System from Siemens Healthcare Diagnostics, Erlangen, Germany. The levels of LDL cholesterol and HDL cholesterol were measured using the SAS-3 Cholesterol Profile kit from Helena Biosciences Europe, Gateshead, UK. Plasma leptin was determined using a commercial ELISA kit from DRG International, Springfield Township, NJ, USA.

Furthermore, plasma levels of insulin, TSH, fT3, fT4, oestradiol, testosterone, Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH), progesterone and vitamin-D were determined using a chemiluminescence immunoassay on the Advia Centaur XP analyzer, also from Siemens Healthcare Diagnostics, Erlangen, Germany. Plasma IGF-1 was analyzed using a chemiluminescence immunoassay on the Immulite 2000 analyzer, also from Siemens Healthcare Diagnostics, Erlangen, Germany. Lastly, circulating free fatty acids (FFA) were measured using a colorimetric kit following the instructions from Wako Chemicals GmbH, Neuss, Germany.

PBMCs isolation from peripheral human blood

PBMCs were isolated from the serum-free blood. The blood was diluted 1:2 with PBS without calcium and magnesium. Five millilitres (mL) of the solution were carefully layered avoiding any mixing in 4 mL of PolymorphPrep solution (Progen) and centrifuged for 30 min at 500 x g for gradient separation. Both cell-containing rings indicative of PBMCs were transferred into a new tube, adding PBS and it was centrifuged for 10 min at 500 x g. The cell pellet was resuspended again with centrifuging for 10 min at 500 x g. This was followed by another fill-up of PBS and centrifuging for 10 min at 500 x g. The cell pellet was resuspended with 750 μL of TRIzol reagent.

SYBR Green One-step RT - qPCR

The extraction of mRNA was carried out by resuspending the sample in TRIzol to which chloroform (VWR chemicals) was added, vortexed and centrifuged at 12000 x g for 15 minutes at 4°C. The aqueous phase was deposited, and the same amount of 70% ethanol (VRW chemicals) was added, both phases being mixed and transferred to a column (RNA extraction kit, Omega Bio Tek). Finally, 40 uL of H20 pure free RNAse was added and centrifuged at 14000 x g for 2 minutes at 21°C.One–step RT – qPCR was carried out in 10 μL reaction volumes containing 10 ng of RNA template, (the RNA concentration was determined spectrophotometrically with Nanodrop (Thermo Scientific NanoDrop), 0.4 μL of 10 μM forward primer, 0.4 μL of 10 μM of reverse primer, 0.4 μL of NZYRT mix, 5 μL One - step NZYSpeedy qPCR Probe master mix (2x) (NYZtech, Lisbon, Portugal) and 1.8 μL of nuclease-free water. In addition, a calibration curve with varying concentrations was used as an amplification control.

After an initial reverse transcription period of 15 min at 50°C, and a polymerase activation of 2,5 minutes at 95°C, the following steps were repeated for 40 cycles in a 96 wells thermal cycler (QuantStudio 3, applied biosystems, Thermo Fisher Scientific): 5 seconds at 95°C and 45 seconds at 60°C followed by a melt curve stage of 15 seconds at 95°C, 1 minute at 60°C and 1 second at 95°C.

The primers employed were designed using exon-boundary-specific gene expression assays using the online tools provided by the PRIMER-blast program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The control housekeeping gene used was β-actin. The list of utilized primers is provided in key resources table.

QUANTIFICATION AND STATISTICAL ANALYSIS

To describe the sample, mean and standard deviation or median and interquartile rank were calculated for the quantitative variables using Pearson's chi-squared test (P), Wilcoxon test (W), T-test (T), or Welch Two Sample t-test (We) (Table 1). Frequencies and percentages were calculated for the qualitative variables. To carry out the comparison between groups, the T-test or Mann-Whitney test and Chi-Squared Test were used (Figure 1). To study the association between *PTK2B* gene expression and biochemical and anthropometric variables Pearson's Correlation was used (Figure 2; Table 2).

To study the main objective, a multivariate logistic regression was performed. The model was constructed from univariate regression analysis with each dependent variable. Candidate variables considered were those whose p-value was less than 0.20, or those considered relevant to the researcher's assessment (Table 3). Subsequently, the final logistic regression model was determined based on the Akaike Information Criterion (AIC) (Figure 3; Table 4).

The association between *PTK2B* gene expression and two age categories (3.5 – 9.3, and 9.4 – 15.3) (Table S1) and the association between *PTK2B* gene expression and BMI Z-score on the puberal stages (Table S3) were studied by Pearson's Correlation.

The development of obesity, the distribution of fat mass (Figure S1 – A), trunk fat mass (Figure S1 – B), waist fat mass (Figure S1 – C), pelvis fat mass (Figure S1 – D), arm fat mass (Figure S1 – E) and leg fat mass (Figure S1 – F) by ages were studied.

To study the association between *PTK2B* gene expression and biochemical and anthropometric variables depending on prepuberal, early puberty, and post puberty by using Pearson's Correlation (Figure S2; Table S2).

Statistical packages used to perform the statistical analysis were: GraphPad Prism software (GraphPad Software, 2023) and R software (R Core Team, 2023), The statistical significance is indicated as follows: *p-value < 0.05, **p-value < 0.01, and ***p-value< 0.001.

ADDITIONAL RESOURCES

This work is not part of/involves a clinical trial.