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Lack of Association Between *PCK1* Polymorphisms and Obesity, Physical Activity, and Fitness in European Youth Heart Study (EYHS)

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Phosphoenolpyruvate carboxykinase-1 (*PCK1*) is the rate-limiting enzyme in the hepatic gluconeogenic pathway. Studies have shown that overexpression of *Pck1* in mice results in obesity-related traits and higher levels of physical activity (PA). Therefore, our aims were to investigate whether common genetic variation in the *PCK1* gene influences obesity-related traits, PA, and fitness, and to examine whether PA and fitness attenuate the influence of the *PCK1* polymorphisms on obesity in children. Analyses were undertaken on data from Danish and Estonian children (958 boys and 1,104 girls) from the European Youth Heart Study (EYHS), a school-based, cross-sectional study of children (mean \pm s.d. age: 9.6 \pm 0.4 years) and adolescents (15.5 \pm 0.5 years). We genotyped eight polymorphisms that captured the common genetic variations in the *PCK1* gene. The association between the *PCK1* polymorphisms and BMI, waist circumference (WC), sum of four skinfolds, PA, and fitness was tested using an additive model adjusted for age, age-group, gender, maturity, and country. Interactions were tested by including interaction terms in the model. None of the polymorphisms were significantly associated with BMI, WC, sum of four skinfolds, PA, and fitness, and also with the risk of being overweight or obese ($P > 0.05$). The interactions between the polymorphisms and age-group, gender, PA, and fitness were not statistically significant. This is the first study to comprehensively examine the association of *PCK1* polymorphisms with obesity, PA, and fitness. Despite strong evidence from animal studies, our study in the EYHS cohort failed to identify an association of *PCK1* polymorphisms with obesity, PA, and fitness.

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INTRODUCTION

Studies have shown that variations in genes that encode enzymes involved in carbohydrate metabolism pathways are associated with an increased risk of obesity and related traits (1–4). One such gene, which may be involved in the pathogenesis of obesity, is the phosphoenolpyruvate carboxykinase-1 (*PCK1*, also known as *PEPCK1*) gene, as it encodes the cytosolic isozyme of phosphoenolpyruvate carboxykinase (5). A recent study showed an association between human *PCK1* mRNA expression in subcutaneous adipose tissue and BMI (6). Besides functioning as a gluconeogenic enzyme catalyzing the conversion of oxaloacetate to phosphoenolpyruvate in the liver and kidney (7), *PCK1* is also a target for peroxisome proliferator-activated receptor- γ co-activator 1 α (*PPARGC1A*) (8), a mediator of adipocyte differentiation and metabolism (9).

Animal studies involving whole body and tissue-specific *Pck1* knockout mice and tissue-specific overexpression of *PCK1* have resulted in obesity, lipodystrophy, and fatty liver (10). Overexpression of *Pck1* was found to result in obesity as a result of increased glyceroneogenesis and fatty acid esterification (11). Transgenic rats overexpressing the *Pck1* gene and lacking its entire upstream regulatory region were found to develop mild glucose intolerance, hyperinsulinemia and were prone to weight gain (12). This effect was further supported by another study, where *Pck1* overexpression in adipose tissue of mice in the presence of high-fat feeding resulted in low levels of energy expenditure, obesity, severe insulin resistance, and type 2 diabetes (13). Recently, it was shown that increased expression of the *Pck1* gene in transgenic mice leads to enhanced capacity to imposed exercise with an increase in the number of mitochondria and content of

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triglyceride in the skeletal muscle (14). This is consistent with the evidence for the paradox in endurance-trained athletes, where skeletal muscle of trained endurance athletes is markedly insulin sensitive and has a high oxidative capacity, despite having elevated lipid content (15). Moreover, transgenic mice ate 60% more but had half the body weight and 10% of the body fat compared to the control mice (14). Thus, it would seem that *PCK1* enhances the storing of energy as fat, which in the presence of positive energy balance or perhaps specifically inactivity is detrimental for health but in the presence of physical activity (PA) leads to enhanced exercise capacity.

Regions near the *PCK1* locus on human chromosome 20 have been implicated in obesity (16). In addition, several studies in humans have shown associations of specific polymorphisms in the *PCK1* gene with type 2 diabetes and related metabolic traits (4,17–20). However, the present study investigates the association of single-nucleotide polymorphisms (SNPs) in the *PCK1* gene with obesity, PA, and fitness and examines the interaction between the *PCK1* SNPs, and PA and fitness on obesity risk in the European Youth Heart Study (EYHS), a large population-based study of children and adolescents from Denmark and Estonia.

METHODS AND PROCEDURES

Study population

The details of the EYHS have been described in detail previously (21–23). In brief, the EYHS is a school-based, cross-sectional study of pre- and early pubertal children randomly selected by a two-stage sampling strategy conducted in four countries: Denmark, Estonia, Norway, and Portugal. Genotypic data are available in two of the EYHS study centers (Denmark and Estonia). A total of 958 boys and 1,104 girls from Denmark and Estonia were included in the analyses (Table 1). These boys and girls were recruited into two age-groups: children (school grade 3) (mean \pm s.d. age: 9.6 ± 0.4 years; range: 8.4–11.3 years) and adolescents (school grade 9) (mean age: 15.5 ± 0.5 years; range: 14.1–17.8 years). The study was approved by the local research ethics committees of each study center and performed in accordance with the Helsinki Declaration. All parents gave written informed consent for their child to participate and all children gave verbal assent.

Measurements

Weight and height were measured using standard techniques with the participants in light clothing and barefoot. BMI was calculated as weight (kg)/height (m²). BMI was standardized according to BMI reference charts derived by the Cole *et al.* LMS method (24). Waist circumference

(WC) in centimeters was measured with a metal anthropometric tape midway between the lower rib margin and the iliac crest, at the end of gentle expiration. The measurements were taken twice, and the mean of the two values was used for further calculations. Four skinfold thickness measurements (triceps, biceps, subscapula, and suprailiac; mm) were taken on the left side of the body in duplicate or triplicate, according to the criteria described by Lohman *et al.* (25), and the two closest measurements at each site were averaged. The sum of skinfolds has been shown to correlate highly with dual-energy X-ray absorptiometry-measured percentage body fat (26). Both inter- and intratester reliability data were recorded for skinfold and waist/hip circumference measurements where the skill of the tester might influence the results. The data suggested that both inter- and intratester variability are within acceptable limits and that it is unlikely that significant between-country testing errors have been introduced (21). Sexual maturity was assessed using the five-stage scale for breast development in girls and pubic hair in boys, according to Tanner (27).

PA was assessed with an MTI ActiGraph (Manufacturing Technology, Fort Walton Beach, FL) accelerometer over two weekdays and two weekend days (28–30). The outcome variable was daily activity (counts/min) that is an indicator of the total amount (average intensity) of PA. This variable was derived by dividing total accelerometer counts/day by the duration the accelerometer was worn on each day, and then averaging across the measurement days. This variable has been shown to be significantly correlated with PA energy expenditure obtained by the doubly labeled water method (31). In addition, fraction of time spent sedentary (<500 counts/min) and at moderate-to-vigorous intensity physical activity (MVPA, >2,000 counts/min) were derived. Our threshold for MVPA corresponds to a walking speed of ~3–4 km/h (30). Before analyses, we excluded all time blocks with ten or more consecutive minutes of zero counts, assuming that the monitor was not worn during that time. On this basis, we included all individuals accumulating at least 600 min/day for at least 3 days including one weekend day. Accelerometric PA data were available for 1,294 children. In addition to the objective measures of PA, inactivity was assessed subjectively by number of hours/week spent on TV viewing. Sleep time was not included in the period of inactivity.

Physical fitness was determined by a maximum cycle ergometer test, as previously described (32). Briefly, the workload was preprogrammed to increase on a computerized cycle ergometer (Monark 839 Ergometric Monark Exercise, Varberg, Sweden) every third minute until exhaustion. Initial workload and increments were 20 or 25 W, depending on whether the body mass of the child was below or above 30 kg. Heart rate was registered continuously (Polar Vantage NV; Polar Electro Oy, Kempele, Finland). Criteria for exhaustion were a heart rate >185 beats/min, failure to maintain a pedaling frequency of at least 30 rpm, and a subjective judgment by the observer that the child could no longer keep up, even after vocal encouragement. All children from one country were tested by the same person. The maximal power output (W_{max}) was calculated as the power in the last fully completed workload plus the power increment of

Table 1 Participant characteristics by gender and age-group in the European Youth Heart Study

Phenotypes	Boys (n = 958)		Girls (n = 1,104)	
	Children (n = 586)	Adolescents (n = 372)	Children (n = 638)	Adolescents (n = 466)
Age (years)	9.7 \pm 0.4	15.5 \pm 0.5	9.6 \pm 0.4	15.5 \pm 0.5
BMI (kg/m ^{2a})	0.25 \pm 1.0	0.22 \pm 0.92	-0.006 \pm 1.1	0.01 \pm 0.96
BMI (kg/m ^{2b})	17.11 \pm 2.2	20.5 \pm 2.55	17.08 \pm 2.6	20.57 \pm 2.7
Waist circumference (cm)	59.1 \pm 1.8	71.1 \pm 5.6	58.0 \pm 6.3	66.6 \pm 5.5
Sum of four skinfolds (mm)	29.91 \pm 11.6	29.9 \pm 12.9	33.6 \pm 15.8	45.2 \pm 16.3
Physical activity (counts/min)	745.6 \pm 11.6 (n = 406) ^c	550.3 \pm 17.9 (n = 175) ^c	615.7 \pm 8.9 (n = 446) ^c	452.7 \pm 10.1 (n = 267) ^c

Values are represented as mean \pm s.d.

^aBMI standardized according to BMI reference charts derived by Cole's least median squares method (22). ^bUncorrected BMI values. ^cNumber of samples for which the accelerometric physical activity data are available.

the last step multiplied by the time proportion completed of the last step. W_{max} was expressed as W/kg body weight (i.e., normalized as a ratio).

SNP genotyping

In order to capture most of the common genetic variations in the *PCK1* gene, we selected seven tag-SNPs using data from the CEU population of the International HapMap project, phase II (HapMap release 19, dbSNP 125). Tag-SNPs were selected using the pairwise correlation between markers ($r^2 > 0.8$; minor allele frequency (MAF) $\geq 5\%$) (<http://www.broad.mit.edu/mpg/tagger/>), as implemented in Haploview v4.0 (<http://www.broad.mit.edu/mpg/haploview>). In addition, we selected one frequently studied SNP (rs28359554) from the literature, amounting to a total of eight SNPs. Of these eight SNPs, one (rs1042521) was excluded from analyses because it did not meet the Hardy–Weinberg equilibrium expectations ($P = 0.02$). Thus, we analyzed 7 SNPs (6 from HapMap and 1 from literature). Although the seven tag-SNPs captured all common (MAF $> 5\%$) variation reported by the HapMap release 19, they capture 50% of the common SNPs (MAF $> 5\%$) reported in the current HapMap release 26. Genotyping of the *PCK1* gene SNPs was performed by Illumina (San Diego, CA) using the Illumina BeadStation custom array (Illumina). The overall call rate for the Illumina genotyping was $> 98\%$. The minor allele frequencies of the SNPs ranged from 0.07 to 0.50 (Supplementary Table S1 online). Pairwise correlations between SNPs ranged from $r^2 = 0.01$ –0.68 (Figure 1).

Statistical analysis

Statistical analyses were conducted using SAS 9.1 for Windows (SAS Institute, Cary, NC). A likelihood ratio test was performed to confirm that the observed genotype distributions were in Hardy–Weinberg equilibrium ($P > 0.01$) (Supplementary Table S1 online). The genotype distribution of six SNPs for Danish and Estonian children were significantly different ($P < 0.05$), and therefore, we adjusted for country in the association analyses and also examined whether the associations were different between Estonians and Danish by testing a SNP \times country

interaction term. The associations between the SNP and BMI, and SNP and PA were tested using generalized linear models assuming an additive effect for each additional minor allele. Interactions between SNP and age-group, gender, or country were also tested by including the respective interaction terms in the model. All models were adjusted for age, age-group, gender, sexual maturity, and country. In addition to the variables mentioned, the association of the SNPs with WC and sum of four skinfolds was adjusted for height, and the association of the SNPs with fitness was adjusted for body weight. Obesity ($n = 107$) and overweight ($n = 312$) were defined as BMI above the 95th and 85th percentiles, respectively, for European children (33). WC and the sum of four skinfolds were log-transformed to obtain normal distribution for analyses; thus, geometric means are reported in the tables.

Haplotype analyses were carried out for seven-marker haplotypes. Haplotypes prevalent at $> 5\%$ were retained for further haplotype analyses. Haplotype trend regression analysis was performed to test for association between common haplotypes and BMI. Haplotype analysis was done using “R” statistical software (version 2.9.2; <http://www.r-project.org>).

Power calculation

We calculated the power of the present study to detect effect sizes for BMI ($\beta = 0.05$ –0.35 kg/m^2 per minor allele), PA ($\beta = 10$ –70 counts/min per minor allele), and fitness ($\beta = 2$ –16 watts per minor allele) for a range of minor allele frequencies (0.05–0.50) using the Quanto v1.1.1 (<http://hydra.usc.edu/gxe>) for a sample size of 2,062 individuals for BMI and 1,294 individuals for PA (Supplementary Figures S1–S3 online).

RESULTS

We investigated the association of the seven SNPs in the *PCK1* gene with obesity-related phenotypes such as BMI, WC and sum of four skinfolds and found that none of the SNPs were associated with any of the obesity-related traits (Table 2). Accordingly, we found no association between these SNPs and the risk of being overweight or obese ($P > 0.05$). In addition, we did not find any evidence of gender-specific effects ($P > 0.05$), age-group-specific effects ($P > 0.05$) or country-specific effects ($P > 0.05$).

We also looked at the association of the *PCK1* gene SNPs with PA, fraction of time spent at MVPA and fitness and found that the SNPs failed to show any association (Table 3). We examined the interaction between the SNPs, and the PA and fitness in our study population and found that none of the interactions between the SNPs and PA on obesity traits were statistically significant ($P > 0.05$). None of the haplotypes showed an association with BMI (Supplementary Table S2 online).

DISCUSSION

In the present study, we examined the association of the seven variants in the *PCK1* gene with obesity-related phenotypes such as BMI, WC and sum of four skinfolds in a cross-sectional study of pre- and early pubertal children from Denmark and Estonia. None of the variants were associated with any of the obesity-related traits. Despite strong evidence from animal studies (11–13), we could not find any evidence for the association of *PCK1* variants with obesity-related phenotypes in children and adolescents. Previous studies have shown association between genetic variation in *PCK1* and the risk of type 2 diabetes and related traits (4,17–20), of which we genotyped four (rs6070157, rs2179706, rs1042531, and rs707555) but found no evidence for association in the EYHS. As this is the

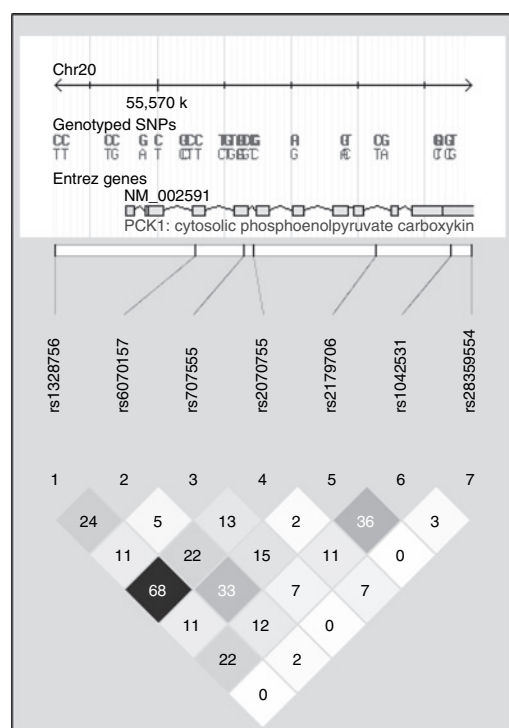


Figure 1 Linkage disequilibrium (LD) plot showing the seven *PCK1* SNPs examined in the European Youth Heart Study. LD values shown in the figure are represented as r^2 . SNP, single-nucleotide polymorphism.

Table 2 Results for association tests between *PCK1* polymorphisms and obesity-related traits

Polymorphisms	Genotypes	N (BMI, WC, sum of four skinfolds)	Standardized BMI (kg/m ²)			Waist circumference (cm)			Sum of four skinfolds (mm)		
			Mean	s.e.	P value ^a	Mean	s.e.	P value ^b	Mean	s.e.	P value ^b
rs1328756 (-1096T/C)	CC	355	0.09	0.05		62.62	0.29		33.31	0.66	
	CT	1,027	0.12	0.03	0.85	62.39	0.17	0.48	33.04	0.39	0.62
	TT	668	0.11	0.04		62.34	0.21		32.89	0.48	
rs6070157 (Ile94Ile)	TT	118	0.11	0.09		62.11	0.51		34.25	1.18	
	TC	801	0.13	0.03	0.61	62.47	0.19	0.89	32.84	0.43	0.71
	CC	1,130	0.10	0.03		62.39	0.16		33.05	0.37	
rs707555 (Leu184Val)	GG	1,536	0.11	0.02		62.44	0.14		33.0	0.31	
	GC	473	0.11	0.04	0.92	62.41	0.25	0.79	33.27	0.57	0.92
	CC	39	0.14	0.15		62.13	0.88		32.07	1.92	
rs2070755 (IVS 4 + 85C/G)	GG	482	0.05	0.04		62.39	0.25		32.51	0.56	
	GC	963	0.13	0.03	0.26	62.46	0.18	0.89	33.12	0.39	0.35
	CC	600	0.12	0.04		62.35	0.23		33.24	0.51	
rs2179706 (IVS 8 + 202T/C)	TT	525	0.09	0.04		62.39	0.24		32.96	0.54	
	TC	1,022	0.15	0.03	0.63	62.51	0.17	0.69	33.15	0.39	0.94
	CC	503	0.06	0.04		62.25	0.25		32.89	0.55	
rs1042531 (3'UTR)	TT	866	0.11	0.03		62.37	0.19		32.91	0.42	
	TG	956	0.11	0.03	0.92	62.47	0.18	0.89	33.03	0.39	0.55
	GG	227	0.10	0.06		62.34	0.37		33.53	0.83	
rs28359554 (3'UTR)	TT	1,776	0.11	0.02		62.39	0.13		33.11	0.29	
	TC	265	0.12	0.06	0.87	62.58	0.34	0.75	32.69	0.75	0.47
	CC	9	-0.18	0.32		61.6	1.82		30.25	3.81	

WC, waist circumference.

^aAdjusted for age, age-group, gender, maturity, and country. ^bAdjusted for age, age-group, gender, maturity, country, and height.

first study examining the association of genetic variants in *PCK1* gene with obesity in young individuals, more studies are needed to explore the association of *PCK1* gene variants with obesity-related phenotypes in other populations.

A recent study showed that increased expression of *Pck1* gene in transgenic mice leads to a high level of PA with an increase in the number of mitochondria, larger triglyceride reserves in their skeletal muscle and enhanced food intake (14). Therefore, to test the hypothesis that variations in the *PCK1* gene are associated with levels of PA, we examined the association of the *PCK1* SNPs with PA and the fraction of time spent at MVPA and found that none of the SNPs showed an association. We also looked at the association of the *PCK1* gene SNPs with fitness and found that the SNPs failed to show any association. In another study, physical exercise reduced hepatic *Pck1* gene expression to ameliorate the insulin resistance in obese Zucker rats and also to improve insulin sensitivity in lean animals (34). Given the role of *PCK1* in contributing to PA in animals, we hypothesized that living a physically active lifestyle would overcome the predisposition to obesity conveyed by *PCK1* gene polymorphisms, despite the fact that the SNPs are not associated with obesity in humans. The reason we pursued this possibility is that gene × environment interactions might conceal a main effect of the environment or genetic polymorphisms on

obesity risk. Therefore, we looked at the interaction between the SNPs and the PA and fitness in our study population. We found that none of the interactions between the SNPs and PA on obesity traits were statistically significant.

Although the results are negative, the findings are of relevance, as it is the first study to examine the association of *PCK1* SNPs with obesity and PA. Importantly, as shown in the **Supplementary Figures S1–S3** online, our study was sufficiently powered to test these hypotheses. Thus, a negative finding is unlikely to be owing to type 2 error. One strength of this study is that we examined the effect of the SNPs on obesity and PA during the early stages of life, when environmental and behavioral factors have had less time to substantially modify the phenotype and therefore, this study on children and adolescents gains importance.

In this study, we failed to show an association of *PCK1* gene SNPs with obesity, PA or fitness, which may be due to the following reasons. We found that, with our sample size, we had 80% power to identify effect sizes for BMI >0.2 kg/m² per minor allele, PA >60 counts/min per minor allele (approximately equivalent to 10–15 min of brisk walking) and physical fitness >11 watts per minor allele at the significance level of 0.05 (**Supplementary Figures S1 and S2** online). If the true effect sizes are lower, we would not be able to detect them.

Table 3 Results for association tests between *PCK1* polymorphisms and physical activity and fitness

Polymorphisms	Genotypes	N (physical activity and MVPA ^a)	N (physical fitness)	Physical activity (counts/min)			MVPA ^a (%)			Physical fitness (absolute watts)		
				Mean	s.e.	P value ^b	Mean	s.e.	P value ^b	Mean	s.e.	P value ^c
rs1328756 (-1096T/C)	CC	228	348	615.11	13.36		8.7	0.3		151.60	1.51	
	CT	636	1,014	616.79	7.97	0.68	8.6	0.2	0.58	152.85	0.88	0.28
	TT	423	659	609.72	9.79		8.5	0.2		153.65	1.09	
rs6070157 (Ile94Ile)	TT	74	117	605.81	23.41		8.4	0.5		149.91	2.59	
	TC	501	794	620.82	8.98	0.63	8.7	0.2	0.77	153.42	0.99	0.76
	CC	712	1,109	610.36	7.53		8.5	0.2		152.74	0.84	
rs707555 (Leu184Val)	GG	966	1,518	619.17	6.45		8.7	0.1		153.02	0.72	
	GC	294	463	600.62	11.69	0.07	8.3	0.2	0.17	152.01	1.30	0.78
	CC	25	38	564.32	40.21		7.9	0.8		160.86	4.54	
rs2070755 (IVS 4 + 85C/G)	GG	304	473	617.46	11.55		8.7	0.2		152.27	1.29	
	GC	607	951	614.89	8.16	0.65	8.6	0.2	0.52	153.87	0.91	0.82
	CC	372	592	610.43	10.45		8.5	0.2		152.07	1.15	
rs2179706 (IVS 8 + 202T/C)	TT	336	517	616.98	10.98		8.6	0.2		152.23	1.23	
	TC	635	1,012	619.15	7.97	0.33	8.7	0.2	0.37	153.43	0.88	0.86
	CC	316	492	601.17	11.31		8.3	0.2		152.50	1.26	
rs1042531 (3'UTR)	TT	549	850	606.19	8.58		8.5	0.2		152.52	0.96	
	TG	603	946	624.24	8.18	0.57	8.8	0.2	0.92	153.62	0.91	0.83
	GG	135	224	601.65	17.29		8.3	0.4		150.81	1.87	
rs28359554 (3'UTR)	TT	1,121	1,750	613.35	5.98		8.6	0.1		152.90	0.67	
	TC	160	263	628.34	15.86	0.87	8.9	0.3	0.88	153.12	1.73	0.86
	CC	6	8	390.35	81.81		4.7	1.7		144.18	9.91	

MVPA, moderate-to-vigorous intensity physical activity.

^aTime fraction spent at moderate- and vigorous-intensity physical activity. ^bAdjusted for age, age-group, gender, maturity, and country. ^cAdjusted for age, age-group, gender, maturity, country, and body weight.

However, although animal studies convincingly illustrated that the *Pck1* gene modulates obesity and PA, it could be that there is, indeed, no effect in humans, either because the gene is not necessary for the regulation of these phenotypes or because functional variation is absent. Another reason could be that the genetic variations within the *PCK1* gene were not captured completely. Although we had chosen the tag-SNPs capturing most of the common genetic variations within the *PCK1* gene based on the International HapMap release 19/phase II (October 2005, on NCBI B34 assembly), we captured only 50% of the common variants (MAF >5%) according to the most recent release (release 26 phase II and III, November 2008, on NCBI B36 assembly, dbSNP b126). As such, we cannot exclude that the absence of association in our study is due to incomplete coverage of the genetic variation in *PCK1*. Finally, the accelerometer variable that was used in the present study may be subject to some random measurement error and therefore likely to affect the precision of the effect estimate in the analysis with PA as outcome (35). In recent genome-wide association studies for obesity-related traits, none of the *PCK1* SNPs reached genome-wide significance for association with BMI (36,37), WC (38,39), and risk of obesity (40).

In conclusion, the data of the present study suggest that the SNPs in the *PCK1* gene are not associated with obesity-related traits, PA, and fitness in Danish and Estonian 10- and 15-year-old children. Furthermore, replication of our finding preferably in an independent larger pediatric cohort with adequate statistical power should be performed in the future to confirm or refute our findings.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

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DISCLOSURE

The authors declared no conflict of interest.

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