Association between *PTPN1* polymorphisms and obesity-related phenotypes in European adolescents: influence of physical activity

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Abstract

Background
To study the associations of Protein Tyrosine Phosphatase-N1 (PTPN1) polymorphisms with obesity-related phenotypes in European adolescents, and the influence of physical activity on these relationships.

Methods
Five polymorphisms of PTPN1 were genotyped in 1,057 European adolescents (12–18 years old). We measured several phenotypes related to obesity, such as adiposity markers, and biochemical and clinical parameters. Physical activity was objectively measured by accelerometry.

Results
The T, A, T, T and G alleles of the rs6067472, rs10485614, rs2143511, rs6020608 and rs968701 polymorphisms, respectively, were associated with lower levels of obesity-related phenotypes (i.e., body mass index, body fat percentage, hip circumference, fat mass index, systolic blood pressure and leptin) in European adolescents. In addition, the TATTG haplotype was associated with lower body fat percentage and fat mass index compared to the AACCA haplotype. Finally, when physical activity levels were considered, alleles of the rs6067472, rs2143511, rs6020608 and rs968701 polymorphisms were only associated with lower adiposity in active adolescents.

Conclusions
PTPN1 polymorphisms were associated with adiposity in European adolescents. Specifically, alleles of these polymorphisms were associated with lower adiposity only in physically active adolescents. Therefore, meeting the recommendations of daily physical activity may reduce obesity risk by modulating the genetic predisposition to obesity.

Impact
- Using gene-phenotype and gene*environment analyses, we detected associations between polymorphisms of the Protein Tyrosine Phosphatase-N1 (PTPN1) gene and obesity-related phenotypes, existing a modulation by physical activity.
- This study shows that genetic variability of PTPN1 is associated with adiposity, while physical activity seems to modulate the genetic predisposition.
- This brings insights about the mechanisms by which physical activity positively influences obesity.

Introduction
Obesity is a major public health problem worldwide, especially in young people. The obese phenotype is likely the result of complex interactions between genetic and environmental factors, its immediate cause being a chronic excess of energy intake over energy expenditure. Several factors can affect energy balance. For example, hunger and satiety influence energy intake, while energy expenditure is influenced by basal metabolism, physical activity, thermoregulation and digestive processes.

Genetic predisposition to obesity can be monogenic, like that caused by genes involved in the control of the appetite and satiety center like leptin or pro-opiomelacortin. Nevertheless, 95% of obesity cases can be explained by multiple genes and complex gene-gene and gene-environment interactions. Physical activity is a known lifestyle factor that affects health status and can modulate genetic risk. Regular physical activity can improve several obesity-related traits like blood pressure, dyslipidemia or sensitivity to insulin. Current evidence suggests that physical activity could attenuate the genetic risk of obesity. For example, a meta-analysis of 200,452 European adults showed that physical activity may attenuate the negative effect of FTO polymorphisms on obesity. In adolescents, we reported that achieving the daily physical activity recommendations (≥ 60 minutes/day of moderate to vigorous physical activity) may reduce the genetic predisposition to obesity associated with the FTO rs9939609, UCP1 rs2071415 and LPL rs1534649 and rs258 polymorphisms.

Protein tyrosine phosphatases (PTPs) are a group of enzymes implicated in the dephosphorylation of tyrosine (Tyr)-phosphorylated proteins. PTPN1, also known as PTPB1, was the first protein-tyrosine-phosphatase discovered, being expressed in several tissues and involved in several signal transduction pathways. PTPN1 plays a key role in insulin signaling by dephosphorylating the insulin receptor and the insulin receptor substrate (IRS-1), which leads to downregulation of its downstream signaling components. PTPN1 also participates in the downregulation of leptin signaling by dephosphorylating molecules upstream in this pathway. In addition, this protein has been related to adiposity, as evidence coming from knockout mice suggests that Ptpn1 deletion provides resistance to weight gain. Several genetic association studies have also associated PTPN1 polymorphisms with obesity-related phenotypes like insulin sensitivity or glucose tolerance. Therefore, single nucleotide polymorphisms (SNPs) of PTPN1 may influence the development of obesity.

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study (www.helenastudy.com) was designed to provide reliable data on nutrition and health-related phenotypes in a relatively large sample of European adolescents from nine different countries. This cohort includes information on polymorphisms of the PTPN1 gene, as well as adiposity markers, and biochemical and clinical parameters related to obesity. Therefore, the aim of this study was to assess the association between PTPN1 polymorphisms and obesity-related phenotypes in European adolescents. We also examined the interaction...
between physical activity and PTPN1 polymorphisms on obesity phenotypes to better understand whether physical activity may modulate the genetic predisposition to develop obesity.

Material And Methods

Participants

The Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) attempted to report the lifestyle and nutritional status of European adolescents. A total of 3865 participants (12–18 year-old) from 10 cities in nine European countries (Austria, Belgium, France, Germany, Greece, Hungary, Italy, Spain, and Sweden) were selected to be part of this study. They were randomly selected from public and private schools in each city between October 2006 and December 2007. Blood samples of one-third of these participants (N = 1155) were collected with the consequent genetic analysis and clinical biochemistry assays. Among these participants, 1057 individuals (552 girls) with data on PTPN1 gene polymorphisms along with adiposity markers, and biochemical and clinical parameters related to obesity were included in this study. From that sample, 698 individuals also included data about objectively measured physical activity. Adolescents and corresponding parents/guardians were fully informed about aims and methods of the study such as inclusion criteria16,19, and signed an informed written consent. The study was conducted according to the Ethical guidelines of the Declaration of Helsinki 1964 (revision of Edinburgh 2000), Good Clinical Practice, and legislation about clinical research in humans in each of the participating countries. Human research committees of each center involved approved the protocol20.

Assessment of adiposity

Weight and height were measured following standard methods21: weight was measured to the nearest 0.1 kg by an electronic scale (SECA 861; Seca Deutschland, Hamburg, Germany) and height to the nearest 0.1 cm by a stadiometer (SECA 225). Waist and hip circumference were measured in triplicate with an anthropometric inelastic tape (SECA 200; Seca Deutschland, Hamburg, Germany). Thereafter, we calculated waist to height and waist to hip ratios, which were used as surrogate measures of central body fat. Body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters. Using BMI (kg/m²), we classified individuals as normal weight, overweight or obesity status according to Cole et al22. Skinfold thickness was measured to the nearest 0.2 mm in triplicate on the left side of the biceps, triceps, subscapularis, suprailium, thigh, and medial calf with a Holtain Caliper (Holtain Ltd, Crymmych, Wales). Body fat percentage was calculated based on skinfold thicknesses (triceps and subcapular) using the equations by Slaughter et al23. Finally, fat mass index (FMI) was calculated by dividing fat mass in kilograms by height in square meters.

Assessment of biochemical and clinical parameters

A total of 30 ml of venous blood was extracted between 8 and 10 am in fasting conditions (ten hours after last meal). Stability of samples regarding transportation between centres had been tested before24. Samples were collected in heparinized and gel tubes for serum, maintained in ice and centrifuged (3,500rpm/15min) within 30 min. After centrifugation they were stored and transported (4–7ºC) to the central laboratory (Bonn, Germany), where they were analyzed directly or after being kept at -80ºC. Serum concentrations of biochemical variables were measured in centralized laboratories.

Total cholesterol (TC), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C), apolipoprotein (apo) A1, apoB, triglycerides (TG) and glucose were analyzed on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) with enzymatic methods. Insulin was measured by a solid-phase two-site chemiluminescent immunometric assay with an Immulite 2000 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany). We computed the homeostatic model assessment (HOMA) as a marker of insulin resistance ([glycaemia X insulin]/22.5). In addition, the quantitative insulin sensitivity check index (QUICKI) was calculated as 1/[(log(insulin) + log(glycaemia)]. Leptin concentrations in duplicate were measured using the RayBio® Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay).

Finally, we measured two clinical parameters: systolic and diastolic blood pressure. Blood pressure was measured on the right arm in an extended position with an automatic oscillometric device (OMRON M6). Adolescents quietly sat for 5 min before the measurements. Two measures were taken 5 min apart, and the mean of both values (in mmHg) was used in analyses.

Assessment of physical activity

Physical activity was assessed during seven consecutive days with a uniaxial accelerometer (GT1M; ActiGraph, Pensacola, Florida) attached to the lower back25. Adolescents were instructed to wear the accelerometer during all time awake and to remove it only during water-based activities. At least 3 days of recording with a minimum of 8 hours registered per day was set as an inclusion criterion25. The time-sampling interval (epoch) was set at 15 seconds. We calculated the time engaged in at least moderate physical activity (≥ 3 metabolic equivalents) based on a standardized cut-off of 2000 counts/min or more25. Moderate to vigorous physical activity (MVPA) was dichotomized into less than 60 min/day (thereafter called inactive adolescents) and 60 min/day or more25 (thereafter called active adolescents) on average.

Genotyping

Blood for DNA extraction was collected in EDTA K3 tubes, stored at the Analytical Laboratory at the University of Bonn and then sent to the Genomic Analysis Laboratory at the Institut Pasteur de Lille (Lille, France). DNA was extracted from white blood cells with the Puregene kit (Qiagen, Courtaboeuf, France) and stored at -20ºC. Samples were genotyped by an Illumina System (Illumina, Inc, San Diego, California) and the software used was GoldenGate (Inc, San Francisco, California). We analyzed tag SNPs that could capture multiple SNPs (R² value > 0.8 according to the HapMap database). A high rate of genotyping success was achieved (≥ 99.9%). 5% of the study sample was systematically analyzed in duplicate and the concordance rate was 100%. Each polymorphism
respected the Hardy-Weinberg equilibrium (HWE; P > 0.05 in all cases; Table 1). Finally, several polymorphisms of the PTPN7 gene showed linkage disequilibrium between them (Supplementary Table S1; Supplementary Figure S1).

### Table 1

<table>
<thead>
<tr>
<th>Major allele</th>
<th>Minor allele</th>
<th>HWE</th>
<th>All centers</th>
<th>Athens Greece</th>
<th>Dortmund Germany</th>
<th>Gent Belgium</th>
<th>Heraklion Crete</th>
<th>Lille France</th>
<th>Roma Hungary</th>
<th>Stockholm Italy</th>
<th>Sweden Austria</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6067472</td>
<td>A</td>
<td>0.61</td>
<td>0.39</td>
<td>0.39</td>
<td>0.37</td>
<td>0.39</td>
<td>0.35</td>
<td>0.39</td>
<td>0.46</td>
<td>0.38</td>
<td>0.43</td>
</tr>
<tr>
<td>rs10485614</td>
<td>A</td>
<td>0.36</td>
<td>0.07</td>
<td>0.09</td>
<td>0.08</td>
<td>0.07</td>
<td>0.09</td>
<td>0.07</td>
<td>0.03</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>rs2143511</td>
<td>T</td>
<td>0.53</td>
<td>0.44</td>
<td>0.44</td>
<td>0.46</td>
<td>0.41</td>
<td>0.49</td>
<td>0.41</td>
<td>0.41</td>
<td>0.42</td>
<td>0.4</td>
</tr>
<tr>
<td>rs6020608</td>
<td>C</td>
<td>0.6</td>
<td>0.29</td>
<td>0.29</td>
<td>0.28</td>
<td>0.29</td>
<td>0.25</td>
<td>0.26</td>
<td>0.36</td>
<td>0.3</td>
<td>0.31</td>
</tr>
<tr>
<td>rs968701</td>
<td>G</td>
<td>1</td>
<td>0.5</td>
<td>0.47</td>
<td>0.52</td>
<td>0.48</td>
<td>0.52</td>
<td>0.5</td>
<td>0.48</td>
<td>0.5</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*The minor allele was determined in the overall sample (hence some MAF are higher than 0.5).*

### Statistical analysis

Deviations from HWE were determined by means of an exact test and considering a p value of 0.05 as a threshold. Associations between polymorphisms, adiposity markers, biochemical and clinical parameters were assessed through generalized linear models. We considered five inheritance models (dominant, recessive, overdominant, codominant, additive). Assuming three genotypes "11", "12" and "22", being "1" the minor and "2" the major alleles, respectively, genotypes were coded in each model as follows: dominant: "11–12" and "22"; recessive: "11" and "12–22"; overdominant: "11–22" and "12"; codominant: "11", "12" and "22"; additive: coded as a numerical variable (2 = "11", 1 = "12", 0 = "22"). These models were used for all analyses except in those where rs10485614 polymorphism was involved. This polymorphism was analyzed using only a dominant model due to the low number of minor homozygotes (Minor allele frequency [MAF] < 0.1; Table 1). Adjustment variables were age, sex and center. In the case of biochemical and clinical variables, BMI was also included as a covariable in the models. We also considered interactions between SNPs and physical activity with the same models but including an interaction term (gene*physical activity). For each polymorphism, p values were computed using a likelihood ratio test (LRT) between a model with the polymorphism/interaction and a null model without it. Associations in which any level (genotype or genotype*physical activity combination) had lower than 10 samples were discarded. These analyses were performed in the R environment using generalized linear models ("stats" R package) and assuming a Gaussian distribution. The response variables (i.e., phenotypes) were log-transformed when the assumption of residuals normality was violated. In the case of obesity/overweight, we used a logistic regression assuming a Binomial distribution. We considered the associations between all SNPs and each phenotype under a given inheritance model as the family test, i.e., the number of tests was equal to the number of SNPs analyzed. We selected those genotypes and phenotypes with certain support for their associations in order to perform interaction and haplotype analyses. In other words, only polymorphisms and phenotypes whose association had certain support were considered for next analyses. Given the exploratory nature of these analyses and the reduced number of independent tests (markers are in linkage disequilibrium, see below), the Bonferroni correction could be too stringent. Instead of this method, we performed an exploratory selection of associations using an approach that controls the expected proportion of false positives (False discovery rate [FDR]) Therefore, we corrected p-values of associations using this approach and then selected those with FDR < 0.1 for the next analyses (interaction and haplotype analyses).

Linkage disequilibrium between polymorphisms and haplotype block structures were evaluated with Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview), along with the "haplo.stats" and "SNPassoc" R packages. First, haplotype blocks were generated by the algorithm of four-gamete rules. For each block, we tested whether the observed frequencies of haplotypes were deviated from that expected under linkage equilibrium. Next, we assessed the association between haplotypes and phenotypes using only additive and dominant models given the low frequency of some haplotypes (Supplementary Table S1). Haplotype-phenotype associations were assessed using a permutation procedure with haplo.stats, controlling in that way for the risk of false positives. This approach generates data under the null hypothesis, that is, no association between obesity-related traits and haplotypes. In each iteration, the values of the phenotype (obesity-related trait) and the covariates are randomly exchanged across individuals, i.e., they are permuted. Given that the phenotype and covariates are shuffled across individuals, but not the genetic data, any signal of association between haplotypes and the phenotype is removed. In other words, we created datasets where there is no association between PTPN7 haplotypes and obesity-related traits, obtaining a null-distribution or control. From these datasets, we obtained the expected level of association between haplotypes and the phenotype just by chance according to the structure of our data. If the null hypothesis is true (i.e., there is no association), changing the combinations of genetic and phenotypic data would not influence the results, i.e., actual and permuted (null) results would be similar. Therefore, the actual results can be compared with a proper null distribution or control, reducing in that way the risk of false positives. The comparison between the actual data and all permutations (at least 10,000) gave a permutation p-value that was used to assess the significance of the association. Therefore, this permutation p-value is a metric about the strength of the evidence in favor of the null hypothesis of no association between haplotypes and phenotypes. We consider enough evidence to support the haplotype-phenotype association in those cases with a permutation p-value < 0.05. We further analyzed these associations in order to assess what specific haplotype is driving the association along with its direction, that is, whether the haplotype is associated with higher or lower phenotypic values compared to other haplotypes. We performed linear regressions between the phenotype and the haplotype levels, which were considered as a categorical variable. The most
frequent haplotype was used as reference; thus, the rest of haplotype levels were compared to this reference haplotype. For each phenotype, several p-values were obtained resulting from the comparison between each haplotype level and the reference haplotype, being these p-values used to calculate the false discovery rate as previously described. We considered FDR < 0.05 as enough evidence to support the existence of differences between the reference and other haplotype levels.

Finally, the interaction analyses between PTPN1 polymorphisms and physical activity were also corrected for multiple comparison. For each phenotype, the p-values obtained for the interaction term between each individual polymorphism and physical activity (see above) were corrected with the False Discovery Rate. We considered a false discovery rate < 0.05 as enough support against the null hypothesis of no interaction.

Results

The characteristics of the study sample are shown in Tables 2 and 3. Characteristics of the specific subset of 698 individuals with physical activity data can be found in Supplementary Tables S2, S3 and S4. These individuals did not differ from the whole cohort in terms of PTPN1 allele frequencies and obesity-related traits. In addition, PTPN1 polymorphisms showed the same power to explain phenotypic variability (R^2) in both datasets (p-value = 0.49 for a Kruskal-Wallis test between groups). Therefore, we found no evidence of sampling bias within the subset of individuals with physical activity data.

<table>
<thead>
<tr>
<th>Center</th>
<th>All</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample size</td>
<td>Overweight (%)</td>
<td>Sample size</td>
</tr>
<tr>
<td>Athens in Greece</td>
<td>104</td>
<td>27.88</td>
<td>45</td>
</tr>
<tr>
<td>Dortmund in Germany</td>
<td>116</td>
<td>26.72</td>
<td>67</td>
</tr>
<tr>
<td>Gent in Belgium</td>
<td>109</td>
<td>6.42</td>
<td>56</td>
</tr>
<tr>
<td>Heraklion in Crete</td>
<td>103</td>
<td>37.86</td>
<td>52</td>
</tr>
<tr>
<td>Lille in France</td>
<td>87</td>
<td>18.39</td>
<td>36</td>
</tr>
<tr>
<td>Pecs in Hungary</td>
<td>137</td>
<td>18.98</td>
<td>53</td>
</tr>
<tr>
<td>Roma in Italy</td>
<td>80</td>
<td>38.75</td>
<td>47</td>
</tr>
<tr>
<td>Stockholm in Sweden</td>
<td>100</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>Vienna in Austria</td>
<td>111</td>
<td>17.12</td>
<td>48</td>
</tr>
<tr>
<td>Zaragoza in Spain</td>
<td>110</td>
<td>18.18</td>
<td>51</td>
</tr>
</tbody>
</table>
The exploratory analysis suggested that the five polymorphisms analyzed were associated with adiposity markers, along with biochemical and clinical parameters related to obesity (rs2143511, rs6020608, rs6067472, rs968701, rs10485614). Table 4 shows associations with FDR < 0.1 under additive and codominant inheritance models. See Supplementary Data 1 for results of the association between all polymorphisms and phenotypes across the five inheritance models considered, including explicative power and minimum sample size (further details also explained in supplementary appendix).
We found that alleles of the rs2143511, rs6020608, rs6067472 and rs968701 polymorphisms were associated with lower adiposity only in active adolescents (i.e., adolescents spending ≥ 60 min/day of moderate to vigorous physical activity). Table 5 shows polymorphism*physical activity interactions with FDR < 0.05 under additive and codominant inheritance models. See Supplementary Data 2 for results of the interaction between physical activity and all polymorphisms across the five inheritance models considered, including explicative power and minimum sample size (further details also explained in supplementary appendix).
Interactions between the studied polymorphisms and physical activity with FDR < 0.05 under additive or codominant models. Mean ± standard deviation is reported for phenotypes in each genotype physical activity level. For each association, it is reported the p-value, FDR and R² under additive and codominant inheritance models. Values are adjusted center, sex, and age (also BMI for biochemical and clinical variables).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Phenotype</th>
<th>11 PI (cm)</th>
<th>12 PI (cm)</th>
<th>22 PI (cm)</th>
<th>11 PA (cm)</th>
<th>12 PA (cm)</th>
<th>22 PA (cm)</th>
<th>Additive P</th>
<th>FDR</th>
<th>R²</th>
<th>Codominant P</th>
<th>FDR</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2143511</td>
<td>Hip circum.</td>
<td>90.95 ± 8.78</td>
<td>91.3 ± 8.43</td>
<td>92.15 ± 8.55</td>
<td>90.07 ± 9.04</td>
<td>91.38 ± 8.62</td>
<td>87.95 ± 8.74</td>
<td>0.029</td>
<td>0.039</td>
<td>0.702</td>
<td>0.011</td>
<td>0.014</td>
<td>1.335</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>20.9 ± 3.54</td>
<td>20.81 ± 3.32</td>
<td>21.26 ± 3.33</td>
<td>21.17 ± 3.17</td>
<td>21.45 ± 3.36</td>
<td>20.18 ± 3.12</td>
<td>0.015</td>
<td>0.029</td>
<td>0.868</td>
<td>0.005</td>
<td>0.012</td>
<td>1.564</td>
</tr>
<tr>
<td></td>
<td>Body fat (%)</td>
<td>24.06 ± 9.52</td>
<td>24.18 ± 8.58</td>
<td>24.66 ± 9.45</td>
<td>21.61 ± 9.62</td>
<td>20.23 ± 9.46</td>
<td>18.85 ± 7.97</td>
<td>0.014</td>
<td>0.028</td>
<td>0.896</td>
<td>0.034</td>
<td>0.068</td>
<td>1.005</td>
</tr>
<tr>
<td></td>
<td>FMI (kg/m²)</td>
<td>5.3 ± 3.04</td>
<td>5.26 ± 2.74</td>
<td>5.48 ± 2.96</td>
<td>4.8 ± 2.87</td>
<td>4.98 ± 2.85</td>
<td>3.96 ± 2.27</td>
<td>0.008</td>
<td>0.017</td>
<td>1.032</td>
<td>0.013</td>
<td>0.026</td>
<td>1.287</td>
</tr>
<tr>
<td>rs6020608</td>
<td>Hip circum.</td>
<td>92.62 ± 8.76</td>
<td>91.36 ± 8.31</td>
<td>91.41 ± 8.65</td>
<td>85.57 ± 8.94</td>
<td>90.05 ± 8.62</td>
<td>90.71 ± 8.79</td>
<td>0.069</td>
<td>0.069</td>
<td>0.488</td>
<td>0.016</td>
<td>0.016</td>
<td>1.208</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>21.62 ± 2.99</td>
<td>20.84 ± 3.26</td>
<td>20.96 ± 3.48</td>
<td>19.46 ± 2.95</td>
<td>21.01 ± 3.04</td>
<td>21.2 ± 3.46</td>
<td>0.103</td>
<td>0.103</td>
<td>0.387</td>
<td>0.012</td>
<td>0.012</td>
<td>1.278</td>
</tr>
<tr>
<td>rs6067472</td>
<td>Hip circum.</td>
<td>93.22 ± 8.04</td>
<td>90.97 ± 8.34</td>
<td>91.49 ± 8.89</td>
<td>87.16 ± 8.8</td>
<td>90.31 ± 8.9</td>
<td>90.68 ± 8.61</td>
<td>0.024</td>
<td>0.039</td>
<td>0.746</td>
<td>0.003</td>
<td>0.01</td>
<td>1.739</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>21.52 ± 3.44</td>
<td>20.74 ± 3.16</td>
<td>21.04 ± 3.58</td>
<td>20.03 ± 3.11</td>
<td>20.97 ± 3.22</td>
<td>21.34 ± 3.39</td>
<td>0.042</td>
<td>0.055</td>
<td>0.605</td>
<td>0.012</td>
<td>0.012</td>
<td>1.281</td>
</tr>
<tr>
<td>rs968701</td>
<td>Body fat (%)</td>
<td>23.27 ± 9.04</td>
<td>24.34 ± 8.78</td>
<td>25.13 ± 9.42</td>
<td>22.13 ± 9.73</td>
<td>20.97 ± 9.39</td>
<td>19.33 ± 7.75</td>
<td>0.005</td>
<td>0.021</td>
<td>1.159</td>
<td>0.019</td>
<td>0.068</td>
<td>1.174</td>
</tr>
<tr>
<td></td>
<td>FMI (kg/m²)</td>
<td>5.05 ± 2.82</td>
<td>5.33 ± 2.84</td>
<td>5.59 ± 2.92</td>
<td>4.94 ± 2.86</td>
<td>4.69 ± 2.8</td>
<td>4.06 ± 2.29</td>
<td>0.003</td>
<td>0.012</td>
<td>1.296</td>
<td>0.013</td>
<td>0.026</td>
<td>1.293</td>
</tr>
</tbody>
</table>

SNP: single nucleotide polymorphism; 1: minor allele; 2: major allele; PI: physically inactive; PA: physically active; P: p-value; FDR: false discovery rate; R²: explicative power in percentage, see supplementary material (Supplementary Data S2) for details about its calculation; additive: additive inheritance model; codominant: codominant inheritance model; BMI: body mass index (calculated as weight in kilograms divided by height in square meters); FMI: fat mass index (calculated as fat mass in kilograms divided by height in square meters).

Physically active adolescents carrying the major T allele of rs2143511 showed lower hip circumference, BMI, body fat percentage, and FMI (Table 5). Regarding the rs6020608 and rs6067472 polymorphisms, physically active adolescents carrying the minor T alleles of these polymorphisms exhibited lower hip circumference and BMI. Finally, physically active adolescents who were carriers of the major G allele of rs968701 also had lower levels of body fat percentage and FMI (Table 5).

Discussion

Our preliminary analyses showed associations of the T, A, T, G alleles of the rs6067472, rs10485614, rs2143511, rs6020608 and rs968701 polymorphisms, respectively, with lower obesity/overweight prevalence and lower values of obesity-related phenotypes (i.e., hip circumference, BMI, body fat percentage, FMI, SBP and leptin). In addition, the haplotype block TATTG constituted by these polymorphisms was associated with lower body fat percentage and FMI. Finally, physical activity may modulate the influence of PTPN1 polymorphisms on adiposity markers in European adolescents. Alleles of rs6067472, rs2143511, rs6020608 and rs968701 polymorphisms were associated with lower adiposity in adolescents meeting the physical activity recommendations.

Previous genetic-association studies have linked PTPN1 polymorphisms with obesity-related phenotypes17, including type 2 diabetes status, total and low-density cholesterol, glucose metabolism, blood pressure and adiposity markers across several Caucasian and Hispanic cohorts34–38. In contrast, other studies have failed to find associations between PTPN1 polymorphisms and obesity-related traits17 like glucose regulation, diabetic status or adiposity in variety of cohorts39,40 including Iranian41 and Pima populations42. With respect to the polymorphisms included in the present study, the rs968701 and rs6020608 have been previously associated with obesity-related phenotypes (glucose regulation and blood pressure), but without considering multiple test correction34–36. In contrast, we found these and other PTPN1 polymorphisms associated with several adiposity markers. Finally, we found no evidence of association for PTPN1 and obesity-related traits in Genome Wide Association Studies (GWAS). This inconsistency can be caused by differences in population-dependent penetration and allele frequencies, as well as subject age, ethnicity and sample size. In the case of GWAS, many more markers are analyzed compared to candidate-gene studies, increasing the risk of false positives and making even more important to control for this type of error. However, some methods for multiple-comparison correction can be too conservative27,29, especially when many genetic markers are analyzed and many of them are in linkage disequilibrium as in the case of GWAS43. In that scenario, a stringent control of false positives may lead to the no detection of genes with smaller effects compared to genes strongly associated with obesity-related traits (e.g., FTO). To our knowledge, there is no previous evidence about the interaction between physical activity and PTPN1 polymorphisms in genetic association studies.

PTPN1 is a negative regulator of the insulin signaling pathway44 as supported by studies showing better glucose homeostasis45 and lower weight gain13 in Ptpn1 knockout mice. Despite the relevance of PTPN1 on insulin signaling, we did not find any association between PTPN1 polymorphisms and phenotypes
related to glucose homeostasis. This could be explained by the differential effect of PTPN1 across tissues. It has been shown that mice with specific Ptpn1 deletion in muscle had improved glucose homeostasis but no differences in weight gain. In contrast, insulin sensitivity did not increase in adipose tissue of whole-body Ptpn1 knockout mice despite the decrease of adiposity. Therefore, PTPN1 protein could have a different function in adipose tissue. Importantly, the associations found between PTPN1 polymorphisms and adiposity in the present study could be explained by the influence of PTPN1 on energy balance, as it has been reported increased resting metabolic rate and energy expenditure in Ptpn1 knockout mice. The impact of PTPN1 on energy balance could be exerted through leptin, given its role in the downregulation of leptin signaling. Leptin regulates energy homeostasis by influencing food intake and energy expenditure. Accordingly, Bence et al. showed increased leptin levels along with reduced food intake and weight in mice with a neuronal-specific Ptpn1 deletion. Our results partially support the influence of PTPN1 on adiposity through leptin, as the AC-CC genotype of rs10485614 was associated with higher leptin levels. In addition, two of the adiposity markers more associated with PTPN1 polymorphisms, fat mass index and body fat percentage, showed a strong positive correlation with leptin (P < 0.0001 and p > 0.6 in both cases; Supplementary Figure S2). These results suggest that leptin resistance could have a relevant role in the patterns of adiposity in this cohort and may be mediated by PTPN1. However, we must bear in mind that rs10485614 was only associated with leptin according to FDR < 0.1 and was not associated with adiposity. This could be caused by the lower number of minor homozygotes for this polymorphism in our cohort, preventing the test of associations under non-dominant models.

PTPN1 could influence energy expenditure not only through leptin, but also through leptin-independent pathways. Several studies have shown increased energy expenditure and decreased adiposity in Ptpn1 knockout mice without differences in food intake. Interestingly, other studies reported lower weight despite existing no differences in food intake for Ptpn1 knockout mice lacking leptin-responsive hypothalamic neurons or with leptin deletion, compared to mice having active Ptpn1. These results suggest a role of PTPN1 on energy expenditure and adiposity independently of leptin in general, and leptin-reduced satiety in particular. The pathways (leptin-dependent or independent) through which PTPN1 favor energy expenditure could implicate the activation of brown adipose tissue (BAT), a relevant tissue on non-shivering thermogenesis. In this regard, it has been shown higher activity of AMP-activated protein kinase (AMPK), a mediator of leptin’s effects, in BAT and muscle tissue of Ptpn1 knockout mice, leading to gene expression changes that enhanced energy expenditure. Similarly, the deletion of Ptpn1 in mice seems to favour BAT mass and activity after cold exposure, along with increase brown adipogenesis (but see Klamann et al. for contrary results). Finally, the influence of PTPN1 on energy homeostasis and adiposity could be mediated by the tropomyosin receptor kinase B (TrkB) and its ligand, the brain-derived neurotrophic factor (BDNF), as both play a key role in the regulation of energy homeostasis. It has been shown that Ptpn1 knockout mice have higher phosphorylation of TrkB and higher increases of core temperature under a treatment of BDNF compared to wildtypes. This suggests that the deletion of Ptpn1 can enhance BDNF-mediated energy expenditure, supporting that PTPN1 could influence energy homeostasis, and consequently adiposity, through different pathways.

Finally, the association of the minor T allele of rs6067472 with lower adiposity and SBP is congruent with results in mice showing a protective effect of PTPN1 deletion against adrenergic hypertension. Bruder-Nascimento et al. reported that Ptn1 knockout mice suffered lower increases of arterial pressure under chronic sympatho-activation. Note, however, that we did not find a strong signal for this association in our study. SBP was associated with PTPN1 polymorphisms only in the first, exploratory analysis (FDR < 0.1), without enough support for the association in further analyses.

We not only found that PTPN1 polymorphisms are associated with obesity-related traits, but also that their influence may be modulated by physical activity. When considering the interaction with physical activity, PTPN1 alleles were associated with less adiposity only in physically active adolescents. These results suggest the existence of a synergy between PTPN1 and physical activity. Evidence coming from mice suggests that physical activity could decrease the content of PTPN1, while Guerra et al. showed an increase of PTPN1 levels in the vastus lateralis of human subjects under bed rest. In addition, it has been shown in mice that aging increases levels of liver PTPN1, adiposity, and the inhibition of insulin signalling. All these changes associated with aging were reverted when mice were exposed to acute exercise. Therefore, PTPN1 variants associated with less content/functionality of PTPN1 in combination with the attenuation of PTPN1 effects by high levels of physical activity may favor a greater reduction of adiposity, that is, they may have a synergistic effect. There are several mechanisms through which the interaction between physical activity and PTPN1 could occur. For example, it could be mediated by Sirtuin 1 (SIRT1). SIRT1 positively impacts insulin resistance through the repression of PTPN1 in cell cultures, being upregulated and having the same effect in the skeletal muscle of old mice exposed to exercise. Therefore, physical activity could inhibit PTPN1 through SIRT1 upregulation. However, we did not find enough support for the association between markers of glucose regulation and PTPN1 polymorphisms. An alternative explanation for the interaction between PTPN1 polymorphisms and physical activity on adiposity could be related to energy expenditure and homeostasis. As previously mentioned, PTPN1 deletion seems to increase mass and activity of BAT and upregulate the BDNF pathway. Interestingly, physical activity could also increase energy expenditure through similar routes. This is congruent with our results showing that PTPN1 polymorphisms were associated with lower adiposity only in physically active adolescents. High levels of physical activity in combination with genetic variants that reduce the functionality or content of PTPN1 may have a synergistic effect on energy expenditure, decreasing adiposity.

All the PTPN1 variants analyzed are intronic, but they could exert an effect on PTPN1 through gene expression. Data from GTEx shows contrasting results for the polymorphisms included in the present study. For example, the rs10485614 polymorphism is associated with lower gene expression for the AA genotype respect to AC. In our study, the AC-CC genotype of rs10485614 was associated with higher leptin levels (FDR = 0.08), which is congruent with the hypothesis that alleles associated with lower PTPN1 levels may be protective regarding obesity-related traits. However, other variants showed opposite results, like the rs2143511 polymorphism. The protective T allele of this polymorphism is associated with higher PTPN1 expression. Note, however, that these results come from whole blood, not adipose or muscle tissue. As previously mentioned, the effect of PTPN1 could vary depending on the tissue. We have not found different expression levels associated with the studied polymorphisms for adipose and muscle tissues, which are the most relevant tissues for interpreting our results. Another possible explanation is that these polymorphisms are in linkage with a coding variant influencing the function of PTPN1. We found certain support for this hypothesis when comparing the explicative power between several models: i) Model including the PTPN1 haplotype; ii) Model including the 5 PTPN1 polymorphisms as independent predictors; iii) Models lacking one of the polymorphisms each time. Each individual variant
showed little independent explicative power (Supplementary Data S3). In other words, each individual polymorphism does not explain much phenotypic variability independently of the rest of variants (further details also explained in supplementary appendix). This suggests that the polymorphisms included in this study are possibly tagging a unique causal variant that could influence the function of PTPN1. Given the lack of data about PTPN1 levels for specific tissues in the present study or cell culture experiments for PTPN1 activity with the studied variants, possible mechanisms of action can only be hypothesized. Therefore, more research is needed to elucidate the specific mechanisms by which these PTPN1 variants and physical activity may influence adiposity and other health markers.

Limitations of the present study should be considered. Firstly, this is a cross-sectional study, hence cause-effect relationships cannot be determined. Our results should be tested in future experimental studies to assess direct causal correlation between PTPN1 polymorphisms and obesity-related phenotypes, along with the interaction with physical activity. In addition, these associations could be modified by gene-gene and other gene-environmental interactions. Finally, we have no information about patterns of relatedness between participants, and we do not know the ethnic/racial origin of the sample. Our results should be considered carefully and studies with larger sample size could help to further support the role of PTPN1 gene on obesity and its interaction with physical activity.

In summary, we found that PTPN1 polymorphisms are associated with adiposity markers in European adolescents. Our results also suggest that the influence of rs6067472, rs2143511, rs6020608 and rs968701 on adiposity markers can be modulated by physical activity. PTPN1 polymorphisms were only associated with lower adiposity in physically active individuals. Therefore, those individuals who meet the recommendations of daily physical activity may have a potential benefit, that is, a reduction of obesity risk by modulating the genetic predisposition to obesity.

**Declarations**

**Data availability statement**

In order to maintain a low risk of re-identification and compliance with the EU General Data Protection Regulation, the clinical and genetic raw data used in this study cannot be public. Interested parties can request the Risk Analysis Assessment approved by the funding agency that includes full explanations about the security measures and their justification.

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**Author contributions**

IL, MGG, MSC, LAM, AGZ, KW, AM, DE, DFST and JRR designed the study; DFST performed all analyses; DFST and JRR wrote the initial draft and all co-authors significantly contributed to the final version.

**Competing interests**

The authors declare that they have no competing interests.

**Consent statement**

As indicated in the Methods section, adolescents and corresponding parents/guardians were fully informed about aims and methods of the study and signed an informed written consent.

**References**


64. Sun, C. et al. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab. 6, 307–19 (2007).
68. GTEx Consortium. GTEx v8 eQTLs: rs10485614 PTPN1 [Internet]. Available from: https://www.gtexportal.org/home/snp/rs10485614
69. GTEx Consortium. GTEx v8 eQTLs: rs2143511 PTPN1 [Internet]. Available from: https://www.gtexportal.org/home/snp/rs2143511

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