Short Communication

Genetic variation in the visfatin (PBEF1/NAMPT) gene and type 2 diabetes in the Greek population

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ABSTRACT

Visfatin (NAMPT formerly known as PBEF1) is an adipokine that is strongly expressed in visceral fat and has caused much debate among researchers, regarding its involvement in glucose homeostasis and insulin resistance. It was initially isolated from bone marrow cells, and its involvement in inflammatory procedures such as sepsis and acute lung inflammation is now evident. Several studies have also reported an association of plasma visfatin levels with obesity. We undertook an initial investigation of the possible association of genetic variants in the NAMPT gene with a mean r^2 of 0.92. No indications of association with disease status were found with any of the tested variants or the inferred haplotypes. Results were also negative when the quantitative traits of weight and BMI were tested. Although our study covers common variants across the population. These three SNPs tag another 12 SNPs over the entire NAMPT gene with a mean r^2 of 0.92. No indications of association with disease status were found with any of the tested variants or the inferred haplotypes. Results were also negative when the quantitative traits of weight and BMI were tested. Although our study covers common variants across the NAMPT gene, the possible involvement of rare variants in T2DM etiology cannot be ruled out and will require the investigation of very large numbers of cases and controls.

1. Introduction

In recent years, an elusive new adipokine was discovered, and initially reported to be preferentially expressed in visceral rather than subcutaneous adipose tissue [1]. This “novel” adipokine was previously known as pre-B-cell colony enhancing factor 1 (PBEF1), and had originally been cloned from bone marrow cells [2]. Furthermore, an enzymatic function has been reported that reveals visfatin/PBEF1 as NAMPT (nicotinamide phosphoribosyltransferase) [3]. It was primarily considered a growth factor that stimulates early B-cell colony formation, synergizing with interleukin-7 (IL-7) and stem cell factor (SCF) [2]. NAMPT (PBEF1) is ubiquitously expressed, and has been shown to play an active role in response to inflammation, with its levels increasing in acute lung inflammation and sepsis [4]. As an adipokine, it was initially postulated [1] that it exerts insulin-mimetic effects, lowering blood-glucose levels in diabetic mice. Since then, the insulin-mimetic action of NAMPT has been severely questioned [1,5] and NAMPT is now thought to participate in obesity and insulin resistance pathology perhaps as an inflammatory protein [6]. However, most related studies agree that plasma NAMPT levels are elevated in obesity [7–9] and T2DM [7,8,10] although results against the involvement of NAMPT in insulin resistance do exist [11,12]. Furthermore, polymorphisms (mostly in the promoter region of the gene), have been associated with plasma glucose concentration at 0 and 120 min during the oral glucose tolerance test (OGTT) in the Chinese and German population [13,14], as well as fasting insulin and glucose in a French-Canadian population [15]. Promoter SNPs have also been associated with lipid metabolism in the Japanese [13,16]. Finally, adding to the debate, one study has found a positive association of a promoter variant of NAMPT with the development of T2DM [17].

2. Methods

We undertook an initial investigation of the possible association of genetic variants in the NAMPT (PBEF1) gene with the development of T2DM in the Greek population. We studied a total of 355 individuals (178 individuals with T2DM and 177 controls). Samples were collected at the Outpatient Clinic of the Diabetes Center at AHEPA University Hospital in Thessaloniki. The study was approved by the local Ethics Committee and informed consent was
obtained from every participating individual. All studied individuals were of Greek origin. Controls were selected among non-diabetic individuals and were matched for age and BMI with our patient group. Thus, there was no statistically significant difference between the average age of cases (mean = 61.2 years of age, standard deviation = 7.96) and controls (mean = 60.2 years of age, standard deviation = 8.96). Similarly, no statistically significant difference was found in the comparison of the average body mass index (BMI) between patients with T2DM (mean = 30.1, standard deviation = 5.87) and non-diabetic individuals (BMI = 29.9, standard deviation = 4.85). DNA was extracted from whole blood using the PUREGENE kit by Qiagen and standard protocols.

In order to select the polymorphic sites in the NAMPT gene that would be genotyped in our sample, we used information from the HapMap database. Although no reference data for the Greek population were available, it has been shown that the HapMap CEPH Europeans constitute a good reference population for inference of the linkage disequilibrium (LD) patterns of other European populations [18,19]. Therefore, we considered this population as reference for SNP selection in our study. The NAMPT gene spans approximately 37 kb over chromosome 7q22.3. We downloaded available genotypes from the HapMap II database and used the algorithm implemented in the software Tagger in order to select tagging SNPs that capture variation over the entire gene. We used an r^2 threshold of 0.8 and applied the multimarker tagging option. Three tagging SNPs were picked for genotyping in our sample; rs2041681 representing SNPs rs10275206, rs4730195, rs4730153, rs3801268, rs2058639, rs3801270 and rs10808150, SNP rs3801272 tagging rs711438, rs10953502, rs3801267 and rs10447822 and SNP rs2098291 representative also of rs13237989. Tagged SNPs were captured with a mean r^2 of 0.92.

Genotyping was performed using the KASPar SNP genotyping system (KBiosciences, Hoddesdon Herts, UK), a novel fluorescence-based allele specific PCR. Allele frequencies were tested for deviations from Hardy–Weinberg equilibrium. The software PLINK [20] was used in order to test for association of each SNP with disease status, using the Cochran–Armitage trend test, as well as allelic and genotypic tests and tests of dominant or recessive gene action. Linear regression, as implemented in PLINK, was also used in order to test for association of SNP alleles and SNP genotypes with weight and BMI. Finally, haplotypes using the three SNPs were inferred and tested both for association with disease status as well as the quantitative traits of weight and BMI.

3. Results

All SNPs complied with Hardy–Weinberg equilibrium distributions. Results of all association tests with disease status are shown in Table 1. There was no difference in allele frequencies between cases and controls. Rarer allele frequencies were 0.32 in cases and 0.33 in controls for SNP rs3801272, 0.43 in both cases and controls for SNP rs2041681, while a small but non-significant difference in frequency between cases and controls is found for SNP rs2098291 (0.29 in cases and 0.26 in controls). No indications of association were found using either the genotypic tests, the Cochran–Armitage test for allelic association, or the model hypotheses of dominant or recessive modes of inheritance. Four common haplotypes for the three SNPs were found and association of the disease with each inferred haplotype was tested. No single haplotype was found associated with disease status (Table 2). Finally, our investigation of association of single SNPs or SNP haplotypes with weight or BMI yielded no statistically significant results in concordance with findings in other studies [13–16]. We would like to point out, that the size of the sample studied, allows the detection of association with variants of large effect, and a larger sample size should be studied in order to test for small effect sizes. Furthermore, a full representation of the LD pattern of the region in the Greek population would only be possible if detailed variation data from the Greek population were available. Nevertheless, as it has been previously shown, the HapMap CEPH Europeans can be successfully used for the selection of tSNPs in other European populations [18,19].

4. Conclusion

Previous studies have reported association of NAMPT variants with fasting insulin, fasting glucose, plasma glucose concentration at 0 and 120 min during the OGTT, as well as serum triglyceride and HDL-cholesterol levels [13–16]. Intriguingly, a recent study showed that NAMPT appears to be a direct contributor to vascular inflammation, a key feature of atherothrombotic diseases linked to metabolic disorders [21]. However, the results of association with T2DM are contradictory [13–17] and the findings we present here do not support a major involvement of the NAMPT gene in the genetic background of T2DM. A larger sample size would increase the power of our study and would allow the investigation of the

Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Test</th>
<th>Affected</th>
<th>Unaffected</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3801272</td>
<td>Genotypic [AA/AG/GG]</td>
<td>17/81</td>
<td>21/75/81</td>
<td>0.72</td>
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<tr>
<td></td>
<td>Cochran–Armitage trend test [A/G]</td>
<td>115/241</td>
<td>117/237</td>
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<td></td>
<td>Dominant [AA + AG]</td>
<td>98/80</td>
<td>96/81</td>
<td>0.88</td>
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<tr>
<td></td>
<td>Recessive [AA/AG + GG]</td>
<td>17/161</td>
<td>21/156</td>
<td>0.48</td>
</tr>
<tr>
<td>rs2098291</td>
<td>Genotypic [TT/CT/CC]</td>
<td>13/79/86</td>
<td>10/72/93</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Cochran–Armitage trend test [T/C]</td>
<td>105/251</td>
<td>92/262</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Dominant [(TT + CT)/CC]</td>
<td>92/86</td>
<td>82/95</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Recessive [TT/CT + CC]</td>
<td>13/165</td>
<td>10/167</td>
<td>0.53</td>
</tr>
<tr>
<td>rs2041681</td>
<td>Genotypic [CC/CT/TT]</td>
<td>32/88/58</td>
<td>30/91/56</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Cochran–Armitage trend test [C/T]</td>
<td>152/204</td>
<td>151/203</td>
<td>0.99</td>
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<tr>
<td></td>
<td>Dominant [(CC + CT)/TT]</td>
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<td>121/56</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Recessive [CC/CT + TT]</td>
<td>32/146</td>
<td>30/147</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 2

Results of haplotype association tests with T2DM status in our sample of 178 patients and 177 controls. Tested haplotypes correspond to rs3801272-rs2098291-rs2041681. Haplotype frequencies for affected and unaffected individuals are shown. The test was implemented using the software PLINK [20].

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Affected</th>
<th>Unaffected</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>0.32</td>
<td>0.33</td>
<td>0.65</td>
</tr>
<tr>
<td>GTC</td>
<td>0.29</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td>GCT</td>
<td>0.28</td>
<td>0.31</td>
<td>0.32</td>
</tr>
<tr>
<td>GCC</td>
<td>0.10</td>
<td>0.096</td>
<td>0.81</td>
</tr>
</tbody>
</table>
possible involvement of NAMPT variants of small effect size in T2DM pathogenesis.

Previous studies focused on the promoter and coding regions of the gene for SNP selection, thus largely overlooking variation in intronic regions of the gene. We, on the other hand, based our study design on the selection of tagging SNPs, thus our results present a picture of association testing for all common variants across the entire gene. The HapMap CEPH European population was used as reference for the selection of SNSP. It should be noted, that, ideally, reference data from the Greek population should be used in order to characterize the LD pattern of the region. However, no such data is available, and it has been previously shown, that CEPH Europeans represent a good reference population for other Europeans, including populations originating from Southern Europe [18,19].

Interestingly, in a recent study, Blakemore et al. [22] found one rare variant in the NAMPT gene to be associated with protection from obesity, with a minor allele frequency of 1.6% in tested controls and a surprising 0% in severely obese children. It is possible that rare variants may also play a role in T2DM susceptibility, however direct sequencing and testing of very large numbers of affected individuals and controls, will be essential in order to investigate the possible involvement of such rare variants both in the NAMPT gene, but also over the entire genome.

Acknowledgments

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The authors hereby declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References