Studies of Metabolic Phenotypic Correlates of 15 Obesity Associated Gene Variants

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Abstract

Aims: Genome-wide association studies have identified novel BMI/obesity associated susceptibility loci. The purpose of this study is to determine associations with overweight, obesity, morbid obesity and/or general adiposity in a Danish population. Moreover, we want to investigate if these loci associate with type 2 diabetes and to elucidate potential underlying metabolic mechanisms.

Methods: 15 gene variants in 14 loci including TME.M18 (rs7561317), SH2B1 (rs7498665), KCTD15 (rs29941), NEGR1 (rs2568958), ET5V (rs7647305), BDNF (rs4923461, rs925946), SEC16B (rs10913469), FAIM2 (rs7138803), GNPD2A (rs10938397), MITCH2 (rs10838738), BAT2 (rs2260000), NPC1 (rs1805081), MAF (rs1424233), and PTER (rs10508503) were genotyped in 18,014 middle-aged Danes.

Results: Five of the 15 gene variants associated with overweight, obesity and/or morbid obesity. Per allele ORs ranged from 1.15–1.20 for overweight, 1.10–1.25 for obesity, and 1.41–1.46 for morbid obesity. Five of the 15 variants moreover associated with increased measures of adiposity. BDNF rs4923461 displayed a borderline BMI-dependent protective effect on type 2 diabetes (0.87 (0.78–0.96, p = 0.008)), whereas SH2B1 rs7498665 associated with nominally BMI-independent increased risk of type 2 diabetes (1.16 (1.07–1.27, p = 7.8×10^-4)).

Conclusions: Associations with overweight and/obesity and measures of obesity were confirmed for seven out of the 15 gene variants. The obesity risk allele of BDNF rs4923461 protected against type 2 diabetes, which could suggest neuronal and peripheral distinctive ways of actions for the protein. SH2B1 rs7498665 associated with type 2 diabetes independently of BMI.

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Introduction

Several studies have established that a genetic contribution to the pathogenesis of obesity exists [1–3]. Since the prevalence of obesity is increasing at alarming rates worldwide, great efforts have been made to identify the genetic components predisposing some individuals to accumulate more body fat. Especially searches for disease-associated single nucleotide polymorphisms (SNPs) have been prioritised, but without noticeable success prior to 2007 where genome-wide association studies (GWAS) were introduced. Several GWAS of body mass index (BMI) and/or obesity have been performed, and the first wave resulted in the suggestion of SNPs in/near the INSL2 [4], FTO [5], PFKP [6], and C7NBL1 [7] genes, however, only SNPs in the FTO locus were convincingly replicated in other GWAS [6,8] and independent replication studies [9–11]. At the end of the first obesity GWAS wave, SNPs near the MC4R gene were identified, both in an independent GWAS [12] and through meta-analysis of several GWAS [13]. These associations have subsequently been confirmed in independent replication studies [14,15]. At the same time, another obesity gene, PCSK1, was suggested using re-sequencing of linkage peaks [16], and some replication studies have established weak associations between this gene and obesity related measures [17,18].

In 2009, the second wave of BMI/obesity GWAS were performed, identifying 15 novel susceptibility loci. A GWAS of BMI and weight in almost 32,000 individuals of primarily European descent validated the associations with FTO and MC4R. Moreover, genome-wide significant associations for variants in/near two genes formerly suggested as biological candidate genes for obesity, BDNF and SH2B1 were observed, together with the identification of seven new BMI/weight loci, TMEM18, KCTD15, NEGR1, SEC16B, ETF5, FAIM2 and BAT2 [19]. A meta-analysis of GWAS on BMI comprising more than 32,000 individuals also confirmed the associations of FTO and MC4R, and furthermore identified six new loci; four of them overlapping with the other BMI/weight GWAS; SH2B1, TMEM18, KCTD15 and NEGR1 and two novel loci GNPDA2 and MITCH2 [20]. The last GWAS of the second wave identified three novel loci, NPC1, MAI and PTER in individuals with early-onset morbid adult obesity [21].

Given the hypothesis-free approach used in GWAS no obvious candidacy could be explained for most of the suggested loci. However, a predominance of the genes nearest the identified SNPs were expressed in the brain, and several particularly in the hypothalamus, suggesting putative roles in the regulation of appetite and energy expenditure [19–20,22].

Since there in the first GWAS wave were identified six loci, but only three FTO, MC4R and PCSK1 were confirmed in independent GWAS or replication studies, the importance of independent replication studies to distinguish between true associations and “winners curse” observations is underlined. Therefore, we attempt to confirm reported GWAS findings of association with obesity in Danish study samples. Furthermore, obesity is a strong risk factor of type 2 diabetes and other key metabolic traits, and therefore, the aim of the present study is also to elucidate whether 15 SNPs in/near the 14 loci from the second obesity GWAS wave associate with type 2 diabetes and other key metabolic correlates.

Results

Confirmation of associations with overweight and obesity

The BDNF rs923461 A-allele associated with increased risk of overweight, with a per allele OR of 1.15 (1.07–1.24, \( p = 2.5 \times 10^{-5} \)), and borderline with obesity with a per allele OR of 1.14 (1.05–1.23, \( p = 0.002 \)) but not with morbid obesity (Table 1, Table S4). This suggested that the effect of the allele might occur at a lower BMI, thus stratifying at 22.5 kg/m\(^2\) showed a higher effect size with an allelic OR of 1.21 (1.09–1.34, \( p = 4.0 \times 10^{-9} \)) (data not shown). Similarly, BDNF rs923464 associated with overweight, with an OR of 1.20 (1.12–1.29, \( p = 1.4 \times 10^{-6} \)), and obesity with an OR of 1.15 (1.06–1.24, \( p = 3.9 \times 10^{-6} \)) but not with morbid obesity. Accordingly, stratifying at 22.5 kg/m\(^2\) showed an OR of 1.27 (1.15–1.40, \( p = 1.8 \times 10^{-6} \)) per T-allele (data not shown).

A different pattern was observed for the TMEM18 rs7561317 G-allele, which strongly associated with obesity with an OR of 1.25 (1.14–1.37, \( p = 2.1 \times 10^{-6} \)) and with morbid obesity with an OR of 1.46 (1.17–1.82, \( p = 8.3 \times 10^{-4} \)) per allele, but not with overweight.

Both the ETF5 rs7647305 C-allele and the GNPDA2 rs10938397 A-allele associated with increased risk of obesity with ORs 1.18 (1.08–1.29, \( p = 1.8 \times 10^{-6} \)) and with morbid obesity with an OR of 1.46 (1.17–1.82, \( p = 1.1 \times 10^{-8} \)) (rs7647305) and 1.15 (1.07–1.23, \( p = 1.1 \times 10^{-6} \)) (rs10938397), but not with overweight or morbid obesity.

Association with type 2 diabetes

Only the SH2B1 rs7498665 G-allele strongly associated with increased risk of type 2 diabetes when adjusting for age and sex with an allelic OR of 1.18 (1.09–1.28, \( p = 3.0 \times 10^{-5} \)) and when additionally adjusting for BMI a nominal association sustained with an OR of 1.16 (1.07–1.27, \( p = 7.8 \times 10^{-5} \)).

The BDNF rs923461 A-allele showed a tendency towards a reduced risk of type 2 diabetes, however, the association was merely nominally, with an OR of 0.87 (0.78–0.96, \( p = 0.006 \)) per allele when adjusting for BMI (Table 1, Table S4), however, when omitting adjustments for BMI the nominally tendency disappeared. The contradictory risk altering patterns of obesity and type 2 diabetes could suggest an interaction between the risk allele and BMI, however, no such interaction was found (data not shown).

Associations with anthropometric measures

The BDNF rs925946 T-allele associated significantly/borderline with three increased measures of adiposity, BMI: 0.35 kg/m\(^2\) (0.18–0.53, \( p = 8.2 \times 10^{-5} \)), waist circumference: 0.76 cm (0.31–1.21, \( p = 8.7 \times 10^{-4} \)), weight 1.05 kg (0.49–1.60, \( p = 2.4 \times 10^{-4} \)) whereas the rs923461 A-allele only associated nominally (Table 2, Table S5). The highest per allele effect sizes of measures of adiposity were observed for the TMEM18 rs7561317 G-allele with increases in BMI of 0.52 kg/m\(^2\) (0.30–0.74, \( p = 2.9 \times 10^{-5} \)), in waist circumference of 1.05 cm (0.50–1.61, \( p = 1.8 \times 10^{-4} \)) and in weight of 1.46 kg (0.77–2.15, \( p = 3.1 \times 10^{-5} \)). Although the A-allele of FAIM2 rs7138803 did not reach statistical significance in the case-control analyses of overweight and obesity, this risk allele strongly associated with increased measures of adiposity with per allele effects on BMI of 0.41 kg/m\(^2\) (0.25–0.57, \( p = 8.2 \times 10^{-5} \)), in waist circumference of 1.06 cm (0.63–1.48, \( p = 4.9 \times 10^{-5} \)) and on weight of 1.41 kg (0.90–1.93, \( p = 7.6 \times 10^{-4} \)) (Table 2, Table S5).

GPNPDA rs10938397 and SEC16B rs10938397 both associated nominally with increased BMI and waist circumference (Table 2, Table S5).

Associations with metabolic traits

BDNF rs925949 associated borderline with elevated levels of circulating leptin, with a 6% (3–10%, \( p = 8.3 \times 10^{-5} \)) per allele increase (ng/ml), unadjusted for BMI (Table 3 (data only shown for BMI adjusted effect sizes), Table S5). Furthermore, this risk allele displayed a tendency towards increased levels of fasting plasma glucose levels, however, associations were merely nominally (Table 4, Table S5). Nominally associations were also
### Table 1. Case-control studies of overweight, obesity and type 2 diabetes in the combined study sample.

| SNP    | Locus | Risk allele | RAf (%) | **Obesity** | **Type 2 diabetes** | OR Without BMI adjustment | p-values |
|--------|-------|-------------|---------|-------------|----------------------|---------------------------|----------|
|       |       |             |         | OR<sub>overweight</sub> | OR<sub>obesity</sub> | OR<sub>morbid obesity</sub> | OR<sub>without BMI adjustment</sub> |           |
| rs7561317 | TMEM18 | G | 84 | 1.08 | 1.25 | 1.46 | 1.13 | 1.04 |
|          |       |       |     | (0.99–1.17) | (1.14–1.37) | (1.17–1.82) | (1.02–1.26) | (0.92–1.17) |
| p       |       |       |     | 0.08 | 2.1 × 10<sup>-4</sup> | 8.3 × 10<sup>-4</sup> | 1.02 | 0.52 |
| rs7498665 | SH2B1 | G | 42 | 1.01 | 1.06 | 1.17 | 1.18 | 1.16 |
|          |       |       |     | (0.95–1.08) | (0.99–1.13) | (1.00–1.36) | (1.09–1.28) | (1.07–1.27) |
| p       |       |       |     | 0.74 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs29941 | KCTD15 | G | 68 | 1.00 | 1.02 | 1.09 | 0.98 | 0.99 |
|          |       |       |     | (0.94–1.07) | (0.95–1.10) | (0.93–1.28) | (0.90–1.07) | (0.90–1.09) |
| p       |       |       |     | 0.93 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs568958 | NEGR1 | A | 60 | 1.01 | 1.04 | 1.09 | 1.08 | 1.06 |
|          |       |       |     | (0.95–1.08) | (0.97–1.11) | (0.93–1.27) | (1.00–1.17) | (0.97–1.16) |
| p       |       |       |     | 0.75 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs764305 | ETV5 | C | 81 | 1.10 | 1.18 | 1.41 | 1.12 | 0.75 |
|          |       |       |     | (1.02–1.19) | (1.08–1.29) | (1.15–1.74) | (1.02–1.24) | (0.93–1.16) |
| p       |       |       |     | 0.02 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs4923461 | BDNF | A | 78 | 1.15 | 1.14 | 1.12 | 0.96 | 0.87 |
|          |       |       |     | (1.07–1.24) | (1.05–1.23) | (0.94–1.35) | (0.87–1.05) | (0.78–0.96) |
| p       |       |       |     | 0.75 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs925946 | BDNF | T | 31 | 1.20 | 1.15 | 1.16 | 0.97 | 0.91 |
|          |       |       |     | (1.12–1.29) | (1.06–1.24) | (0.98–1.37) | (0.89–1.06) | (0.83–1.00) |
| p       |       |       |     | 0.75 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs10913469 | SEC16B | C | 21 | 1.04 | 1.11 | 1.11 | 0.97 | 0.90 |
|          |       |       |     | (0.96–1.12) | (1.03–1.21) | (0.93–1.34) | (0.88–1.06) | (0.81–1.00) |
| p       |       |       |     | 0.32 | 8.0 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs7138803 | FAIM2 | A | 41 | 1.03 | 1.09 | 1.19 | 1.08 | 1.06 |
|          |       |       |     | (0.97–1.10) | (1.02–1.17) | (1.02–1.38) | (1.00–1.17) | (0.97–1.15) |
| p       |       |       |     | 0.33 | 8.0 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs10938397 | GNPDA2 | G | 41 | 1.09 | 1.15 | 1.20 | 1.06 | 0.99 |
|          |       |       |     | (1.02–1.16) | (1.07–1.23) | (1.03–1.14) | (0.97–1.14) | (0.90–1.08) |
| p       |       |       |     | 0.01 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs10838738 | MITCH2 | G | 35 | 1.00 | 1.01 | 0.99 | 1.04 | 1.03 |
|          |       |       |     | (0.94–1.07) | (0.94–1.08) | (0.85–1.17) | (0.96–1.13) | (0.94–1.13) |
| p       |       |       |     | 0.91 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs2260000 | BAT2 | T | 64 | 1.06 | 1.01 | 1.05 | 0.93 | 0.91 |
|          |       |       |     | (0.99–1.14) | (0.94–1.09) | (0.90–1.24) | (0.85–1.01) | (0.83–1.00) |
| p       |       |       |     | 0.10 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs1805081 | NPC1 | A | 58 | 1.06 | 1.10 | 1.21 | 1.09 | 1.07 |
|          |       |       |     | (1.00–1.13) | (1.03–1.18) | (1.03–1.41) | (1.01–1.18) | (0.98–1.17) |
| p       |       |       |     | 0.07 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs1424233 | MAF | G | 47 | 1.05 | 1.06 | 1.07 | 0.97 | 0.94 |
|          |       |       |     | (0.99–1.12) | (0.99–1.14) | (0.92–1.25) | (0.89–1.05) | (0.86–1.02) |
| p       |       |       |     | 0.11 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |
| rs10508503 | PTER | T | 8 | 0.94 | 0.99 | 1.07 | 0.93 | 0.91 |
|          |       |       |     | (0.84–1.05) | (0.87–1.12) | (0.82–1.40) | (0.81–1.07) | (0.78–1.07) |
| p       |       |       |     | 0.29 | 1.9 × 10<sup>-4</sup> | 8.0 × 10<sup>-4</sup> | 1.01 | 0.25 |

Data are presented as odds ratios (OR) (CI 95%) and p-values. OR and p-values are specified for the additive model and adjusted for age and gender.

*Association with type 2 diabetes are additionally adjusted for BMI. RAf: risk allele frequency.

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| SNP     | Locus | Risk allele | RAF (%) | Measures of adiposity | Weight (kg) | Waist-to-hip ratio |
|---------|-------|-------------|---------|-----------------------|-------------|-------------------|
| rs7561317 | TMEM18 | G           | 83      | 0.52                  | 1.05        | 1.46              | 0.003           |
|         |       |             |         | (0.30–0.74)           | (0.50–1.61) | (0.77–2.15)       | (0.0002–0.006)  |
| rs7498665 | SH2B1  | G           | 41      | 0.08                  | 0.23        | 0.24              | 0.001           |
|         |       |             |         | p = 2.9 × 10^{-6}     | p = 1.8 × 10^{-4} | p = 3.1 × 10^{-5} | p = 0.04        |
|         |       |             |         | (−0.09–0.24)          | (−0.19–0.64) | (−0.25–0.78)      | (−0.001–0.003)  |
| rs29941  | KCTD15 | G           | 68      | 0.16                  | 0.29        | 0.53              | 0.0008          |
|         |       |             |         | p = 0.08              | p = 0.19    | p = 0.06          | p = 0.49        |
| rs2568958 | NEGR1  | A           | 59      | 0.06                  | 0.17        | 0.20              | 0.002           |
|         |       |             |         | p = 0.46              | p = 0.42    | p = 0.44          | p = 0.09        |
| rs7647305 | ETV5   | C           | 80      | 0.22                  | 0.70        | 0.69              | 0.003           |
|         |       |             |         | (0.01–0.42)           | (0.18–1.22) | (0.04–1.34)       | (0.0005–0.006)  |
| rs4923461 | BDNF   | A           | 78      | 0.29                  | 0.61        | 0.89              | 0.0007          |
|         |       |             |         | p = 0.04              | p = 0.008   | p = 0.04          | p = 0.02        |
|         |       |             |         | (0.10–0.49)           | (0.11–1.11) | (0.26–1.51)       | (−0.002–0.003)  |
| rs925946 | BDNF   | T           | 31      | 0.35                  | 0.76        | 1.05              | 0.001           |
|         |       |             |         | (0.18–0.53)           | (0.31–1.21) | (0.49–1.60)       | (−0.009–0.004)  |
| rs10913469 | SEC16B | C           | 21      | 0.33                  | 0.83        | 0.90              | 0.003           |
|         |       |             |         | (0.14–0.53)           | (0.33–1.33) | (0.27–1.52)       | (0.00004–0.005) |
|         |       |             |         | p = 9.2 × 10^{-4}     | p = 0.001   | p = 0.005         | p = 0.05        |
| rs7138803 | FAIM2  | A           | 40      | 0.41                  | 1.06        | 1.41              | 0.003           |
|         |       |             |         | (0.25–0.57)           | (0.65–1.48) | (0.90–1.93)       | (0.001–0.005)   |
| rs10938397 | GNPDA2 | G           | 41      | 0.28                  | 0.61        | 0.73              | 0.002           |
|         |       |             |         | p = 8.2 × 10^{-7}     | p = 4.9 × 10^{-7} | p = 7.6 × 10^{-5} | p = 0.005   |
|         |       |             |         | (0.11–0.44)           | (0.19–1.03) | (0.21–1.26)       | (−0.003–0.004)  |
|         |       |             |         | p = 0.001             | p = 0.004   | p = 0.006         | p = 0.1         |
| rs10838738 | MTCH2  | G           | 35      | −0.04                 | 0.007       | −0.12             | −0.004          |
|         |       |             |         | (−0.21–−0.13)         | (−0.43–0.44) | (−0.66–0.42)      | (−0.003–0.002)  |
| rs2260000 | BAT2   | T           | 63      | 0.07                  | 0.29        | 0.58              | 0.0004          |
|         |       |             |         | (−0.09–0.24)          | (−0.14–0.71) | (0.05–1.10)       | (−0.002–0.003)  |
|         |       |             |         | p = 0.39              | p = 0.18    | p = 0.03          | p = 0.74        |
| rs1805081 | NPC1   | A           | 57      | 0.11                  | 0.25        | 0.19              | 0.002           |
|         |       |             |         | (−0.05–0.28)          | (−0.17–0.66) | (−0.33–0.71)      | (−0.0002–0.004) |
| rs1424233 | MAF    | G           | 47      | 0.15                  | 0.16        | 0.44              | −0.0004         |
|         |       |             |         | (−0.01–0.31)          | (−0.25–0.58) | (−0.07–0.95)      | (−0.003–0.002)  |
|         |       |             |         | p = 0.07              | p = 0.44    | p = 0.09          | p = 0.72        |
| rs10508503 | PTER   | T           | 9       | 0.07                  | 0.06        | 0.33              | −0.002          |
|         |       |             |         | (−0.22–0.36)          | (−0.68–0.79) | (−0.58–1.24)      | (−0.006–0.002)  |
|         |       |             |         | p = 0.63              | p = 0.88    | p = 0.48          | p = 0.4         |

Data are presented as effect sizes (CI 95%) per allele and p-values. Effect sizes are given in absolute values and p-values are specified for the additive model and adjusted for age and gender. RAF, risk allele frequency.

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| SNP       | Locus | Triglycerides (mmol/l) | Total cholesterol (mmol/l) | HDL-cholesterol (mmol/l) | LDL-cholesterol (mmol/l) | Leptin | Adiponectin | CRP |
|-----------|-------|------------------------|---------------------------|--------------------------|-------------------------|--------|-------------|-----|
| rs7561317 | TMEM18 | 0.005 | 0.02 | 0.001 | 0.04 | 0.03 | 0.004 | 0.03 |
| rs7498665 | SH2B1 | 0.01 | 0.01 | 0.008 | 0.003 | 0.004 | 0.004 | 0.006 |
| rs29941   | KCTD13 | 0.009 | 0.02 | 0.00008 | 0.002 | 0.001 | 0.0005 | 0.001 |
| rs2568958 | NEGR1 | 0.01 | 0.03 | 0.008 | 0.03 | 0.01 | 0.02 | 0.002 |
| rs7647305 | ETVS | 0.01 | 0.03 | 0.008 | 0.03 | 0.01 | 0.01 | 0.02 |
| rs4923461 | BDNF | 0.01 | 0.01 | 0.005 | 0.03 | 0.03 | 0.008 | 0.002 |
| rs925946  | BDNF | 0.02 | 0.01 | 0.009 | 0.01 | 0.02 | 0.02 | 0.04 |
| rs10913469| SECT16B | 0.008 | 0.02 | 0.004 | 0.02 | 0.03 | 0.02 | 0.01 |
| rs7138803 | FAIM2 | 0.001 | 0.01 | 0.001 | 0.01 | 0.006 | 0.01 | 0.03 |
| rs10938397| GNPOA2 | 0.01 | 0.03 | 0.008 | 0.02 | 0.005 | 0.01 | 0.02 |
| rs10838738| MTCHE2 | 0.002 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.0007 |
| rs2260000 | BAT2 | 0.02 | 0.007 | 0.01 | 0.02 | 0.005 | 0.0005 | 0.02 |
observed for the FAIM2 rs7138803 A-allele which with elevated levels of circulating leptin as well as lower levels of HDL-cholesterol (Table 3, Table S5).

Discussion

In the second obesity GWAS wave 15 variants in/near 14 loci were identified, and several independent studies have already attempted replication of these findings. Here in our Danish study population we aim to validate these associations, and to elucidate whether some of the variants associate with other clinically relevant phenotypes. Our analyses show the same trends towards increased obesity risk, for all variants except rs10508503 near MTCH2.

Other studies have also confirmed the associations between quantitative measures of obesity, i.e. BMI, weight and waist circumference, and/or the risk of obesity for variants in/near TMEM18 [23–27], SEC16B [27], NECR1 [24,27–29], SH2B1 [26,28,30], MTCH2 [24,28,29], GNPDA2 [25,28,30], FAIM2 [25,27,30], BDNF [25,27,29], and KCTD15 [29,30], however, no convincing pattern exists between the verified variants in the performed studies, which could be caused by low power for detecting modest effect sizes, and therefore, as the number of independent replication studies increases, a meta-analyses may be needed to determine true positive associations. The risk of type 2 diabetes has only been addressed in two other studies, and here association between TMEM18 [28], GNPDA2 [28,30], ETV5, FAIM2 and SH2B1 [30] and a BMI-dependent increased risk of type 2 diabetes have been reported. Hence, we are the first to report a tendency towards an increased risk of type 2 diabetes independent of BMI for SH2B1, and rs7498665 could therefore be suggested as a type 2 diabetes variant if this association is replicated in other independent studies. Particularly surprising from our analyses was the finding that the obesity risk allele of BDNF (rs4923461) was borderline associated with a reduced risk of type 2 diabetes when adjusted for BMI. However, these divergent associations were not explained by an interaction between the variant and BMI.

Interestingly, both SH2B1 and BDNF are obvious candidate genes for metabolic disorders based on the biological roles of the encoded proteins. That is, the SH2B1 adaptor protein is involved in several signal transduction processes, including the signalling mediated by the binding of insulin and leptin [31]. The association with type 2 diabetes is in line with previous studies showing insulin resistance in knockout mice [32–35]. Furthermore, a recent study demonstrated that TgKO mice, that only express SH2B1 in the brain and thus have loss of peripheral SH2B1, have impaired insulin sensitivity independent of body weight [35]. However, none of the examined measures of glucose homeostasis supported a possible reduction in insulin sensitivity in our study.

### Table 3. Cont.

| SNP     | Locus | Fasting serum lipids (mmol/l) | Fasting serum adipokines and CRP (ng/ml) |
|---------|-------|-------------------------------|----------------------------------------|
|         |       | Triglycerides | Total cholesterol | HDL-cholesterol | LDL-cholesterol | Leptin | Adiponectin | CRP |
| rs1805081 | NPC1  | p = 0.05        | p = 0.63       | p = 0.03       | p = 0.31       | p = 0.84       | p = 0.88       | p = 0.48       |
| rs1424233 | MAF   | p = 0.12        | p = 0.02       | p = 0.63       | p = 0.74       | p = 0.44       | p = 0.43       | p = 0.54       |
| rs10508503 | PTER  | p = 0.02        | p = 0.50       | p = 0.03       | p = 0.43       | p = 0.69       | p = 0.97       | p = 0.46       |

Data are presented as effect sizes (95% CI) per allele and p-values.

*Effect sizes are adjusted for age, sex and BMI and given as absolute values for total cholesterol and HDL-cholesterol, LDL-cholesterol, and as percentages for triglyceride, leptin, adiponectin and CRP since these traits are logarithmically transformed. LDL-cholesterol is calculated as: ((total cholesterol – HDL-cholesterol – triglyceride) / protein. **p-values are specified for the additive model and adjusted for age, sex and BMI. CRP, C-reactive protein.

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| Measures of glucose homeostasis | SNP     | Locus | Fasting plasma glucose (mmol/l) | Fasting serum insulin (pmol/l) | HOMA-IR (mmol/l×pmol/l) | Insulinogenic index (pmol×pmol⁻¹) |
|--------------------------------|---------|-------|--------------------------------|-------------------------------|------------------------|----------------------------------|
| rs7561317                      | TMEM18  | 0.001 | 0.03                           | 0.03                          | 0.03                   | 0.02                             |
|                                |         |       | ([−0.004−0.007])               | ([−0.06−0.005])               | ([−0.05−0.002])        | ([−0.05−0.001])                 |
|                                |         |       |      *p = 0.58                  |      *p = 0.02                 |      *p = 0.04          |      *p = 0.28                   |
|                                |         |       |      p = 0.06                   |      p = 0.65                  |      p = 0.42           |      p = 0.95                    |
| rs7498665                      | SH2B1   | 0.0002| 0.005                          | 0.006                         | 0.006                  | 0.006                            |
|                                |         |       | ([−0.004−0.004])              | ([−0.13−0.02])               | ([−0.01−0.02])         | ([−0.17−0.03])                 |
|                                |         |       |      *p = 0.94                 |      *p = 0.56                 |      *p = 0.57          |      *p = 0.62                   |
|                                |         |       |      p = 0.73                   |      p = 0.34                 |      p = 0.33           |      p = 0.50                    |
| rs29941                        | KCTD15  | 0.0002| 0.005                          | 0.007                         | 0.003                  | 0.003                            |
|                                |         |       | ([−0.002−0.006])              | ([−0.01−0.02])               | ([−0.01−0.03])        | ([−0.02−0.03])                 |
|                                |         |       |      *p = 0.42                 |      *p = 0.62                 |      *p = 0.51          |      *p = 0.80                   |
|                                |         |       |      p = 0.20                   |      p = 0.20                 |      p = 0.15           |      p = 0.61                    |
| rs2568958                      | NGR1    | 0.003 | −0.02                          | −0.01                         | −0.02                  | −0.02                            |
|                                |         |       | ([−0.0005−0.007])           | ([−0.05−0.002])               | ([−0.03−0.007])       | ([−0.05−0.002])                 |
|                                |         |       |      *p = 0.09                 |      *p = 0.08                 |      *p = 0.21          |      *p = 0.04                   |
|                                |         |       |      p = 0.06                   |      p = 0.27                 |      p = 0.52           |      p = 0.07                    |
| rs7647305                      | ETV5    | 0.0008| 0.003                          | 0.004                         | 0.002                  | 0.002                            |
|                                |         |       | ([−0.004−0.006])              | ([−0.02−0.03])               | ([−0.02−0.03])        | ([−0.03−0.03])                 |
|                                |         |       |      *p = 0.74                 |      *p = 0.79                 |      *p = 0.78          |      *p = 0.89                   |
|                                |         |       |      p = 0.36                   |      p = 0.24                 |      p = 0.22           |      p = 0.72                    |
| rs4923461                      | BDNF    | −0.003| −0.01                          | −0.01                         | −0.01                  | 0.01                             |
|                                |         |       | ([−0.007−0.002])              | ([−0.03−0.01])               | ([−0.04−0.01])        | ([−0.02−0.04])                 |
|                                |         |       |      *p = 0.26                 |      *p = 0.41                 |      *p = 0.34          |      *p = 0.38                   |
|                                |         |       |      p = 0.84                   |      p = 0.50                 |      p = 0.52           |      p = 0.13                    |
| rs925946                       | BDNF    | 0.004 | −0.01                          | −0.008                        | −0.009                 | −0.009                           |
|                                |         |       | ([−0.0002−0.008])            | ([−0.03−0.008])               | ([−0.03−0.01])       | ([−0.03−0.02])                 |
|                                |         |       |      *p = 0.06                 |      *p = 0.22                 |      *p = 0.47          |      *p = 0.48                   |
|                                |         |       |      p = 0.003                  |      p = 0.40                 |      p = 0.18           |      p = 0.87                    |
| rs10913469                     | SEC16B  | 0.001 | −0.02                          | −0.02                         | 0.009                  | 0.009                            |
|                                |         |       | ([−0.003−0.006])              | ([−0.04−0.006])               | ([−0.04−0.008])       | ([−0.02−0.04])                 |
|                                |         |       |      *p = 0.60                 |      *p = 0.14                 |      *p = 0.19          |      *p = 0.52                   |
|                                |         |       |      p = 0.14                   |      p = 0.71                 |      p = 0.56           |      p = 0.16                    |
| rs7138803                      | FAIM2   | −0.0005| −0.002                         | −0.002                        | 0.006                  | 0.006                            |
|                                |         |       | ([−0.004−0.003])              | ([−0.02−0.02])               | ([−0.02−0.02])       | ([−0.02−0.03])                 |
|                                |         |       |      *p = 0.79                 |      *p = 0.82                 |      *p = 0.82          |      *p = 0.63                   |
|                                |         |       |      p = 0.23                   |      p = 0.02                 |      p = 0.02           |      p = 0.09                    |
| rs10938397                     | GNPD2   | −0.001| 0.006                          | 0.004                         | 0.01                   | 0.01                             |
|                                |         |       | ([−0.005−0.003])              | ([−0.01−0.02])               | ([−0.02−0.02])       | ([−0.01−0.04])                 |
|                                |         |       |      *p = 0.51                 |      *p = 0.56                 |      *p = 0.70          |      *p = 0.28                   |
|                                |         |       |      p = 0.72                   |      p = 0.03                 |      p = 0.04           |      p = 0.08                    |
| rs10838738                     | MTC1H2  | −0.0002| −0.01                          | −0.01                         | 0.007                  | 0.007                            |
|                                |         |       | ([−0.004−0.004])              | ([−0.03−0.007])               | ([−0.03−0.008])       | ([−0.03−0.02])                 |
|                                |         |       |      *p = 0.94                 |      *p = 0.21                 |      *p = 0.22          |      *p = 0.56                   |
|                                |         |       |      p = 0.82                   |      p = 0.18                 |      p = 0.19           |      p = 0.45                    |
| rs2260000                      | BAT2    | −0.003| 0.0003                         | −0.004                        | −0.001                 | −0.001                           |
|                                |         |       | ([−0.007−0.0009])             | ([−0.02−0.02])               | ([−0.02−0.02])       | ([−0.02−0.02])                 |

| Table 4. Effects sizes of the obesity risk alleles on measures of glucose homeostasis, with and without adjustments for BMI in the population-based Inter99 cohort. |
BDNF, brain-derived neurotrophic factor, is implicated in the regulation of body weight [36]. Yet, our results could suggest more ways for action of the BDNF protein. This is also supported by two studies of db/db mice. One where exogenous BDNF treatment reduces blood glucose concentrations independently of the hypophagic effect [37] and another where subcutaneous BDNF resulted in reduced blood glucose levels in BDNF administered mice, whereas pair-fed control mice displayed unchanged levels, despite that plasma insulin levels were significantly reduced in both groups [38].

Consistent with animal models, plasma BDNF levels have previously been reported to be inversely correlated with fasting plasma glucose among type 2 diabetes patients and to be associated with the severity of insulin resistance [39]. This suggests that BDNF regulates blood glucose homeostasis and insulin sensitivity peripherally. Hence, tissue specific up-regulation of endogenous BDNF levels in peripheral tissues could explain this BMI-dependent protective effect on type 2 diabetes for the reported BDNF obesity risk allele (rs925946).

Contradictory, quantitative trait analyses show that the other risk allele near BDNF (rs925946) associated with elevated and not decreased fasting plasma glucose levels in our study population. Serum leptin and the inflammatory marker CRP were also found to be significantly elevated among rs925946 T-allele carriers in the present study. However, these associations omitted when adjusting for BMI and is therefore most likely mediated through the increased body fat accumulation, rather than being the cause of it.

Different neuronal and peripheral regulatory mechanisms of BDNF could be explained by different isoforms of the protein, and in fact tissue specific alternative splicing has been reported for BDNF in humans [40,41]. Furthermore, the BDNFOS gene, which is a non-protein-coding natural antisense transcript positioned downstream of BDNF in reverse orientation, has been suggested to have an important role in tissue specific regulation of BDNF expression through the formation of dsRNA duplexes [41]. In fact both gene variants are positioned within the BDNFOS locus. Thus, it could be hypothesized that the risk alleles could result in a muscle specific impairment of BDNFOS transcription and splicing, which would lead to a reduction in complementary BDNF RNAs and consequently increase the level of BDNF. Therefore physiological and experimental studies to illuminate the tissue specific and functional role of BDNF and BDNFOS will be of great interest.

Of the remaining associated obesity risk loci the potential functional role is less obvious as little is known of the encoded proteins. Furthermore, some of the identified risk variants are next to additional loci, in which the risk variants may influence as well. However, most of the identified loci are highly expressed in the brain, particularly in the hypothalamic region, indicating roles in appetite regulation and energy expenditure [19,20,22].

Many analyses were made in this study and some should therefore be regarded as hypothesis-generating with confirmation in independent studies as an important next step, but these analyses in the population-based Inter99 cohort still contribute to the follow-up of the reported findings of the second obesity GWAS wave.

It should moreover be noted that individuals from the Inter99 cohort (Note S1) were included in the follow-up of the top 43 variants in the BMI GWAS performed by Thorleifsson et al. However, since these studies were pooled with other ethnicities, we found it necessary to elucidate whether the associations with type 2 diabetes and obesity were present in an ethnically homogeneous study to avoid confounding by population stratification. In view of that, little overlap is observed in associations with type 2 diabetes suggesting that the effect of the BMI risk variants on type 2 diabetes risk is dependent on geographic origin. Moreover, we have in the present study examined the underlying metabolic phenotypes profoundly in order to shed light on the possible metabolic mechanisms causing the reported associations. The Inter99 cohort is in addition used in the replication part of the

Table 4. Cont.

| SNP        | Locus | Fasting plasma glucose (mmol/l) | Fasting serum insulin (pmol/l) | HOMA-IR (mmol/lxpmol/l) | Insulinogenic index (pmolxpmol⁻¹) |
|------------|-------|--------------------------------|-------------------------------|-------------------------|-----------------------------------|
| rs1805081  | NPC1  | 0.0008                         | -0.01                         | -0.01                   | 0.01                              |
|            |       | (-0.003–0.005)                 | (-0.03–0.004)                 | (-0.03–0.005)           | (-0.009–0.04)                     |
|            |       | *p=0.70                        | *p=0.13                       | *p=0.15                 | *p=0.24                           |
|            |       | p=0.45                         | p=0.45                        | p=0.52                  | p=0.24                            |
| rs1424233  | MAF   | -0.001                         | -0.001                        | -0.002                  | 0.02                              |
|            |       | (-0.005–0.003)                 | (-0.02–0.02)                  | (-0.02–0.02)            | (-0.003–0.04)                     |
|            |       | *p=0.56                        | *p=0.90                       | *p=0.85                 | *p=0.09                           |
|            |       | p=0.99                         | p=0.41                        | p=0.42                  | p=0.04                            |
| rs10508503 | PTER  | -0.002                         | -0.02                         | 0.01                    | -0.02                             |
|            |       | (-0.009–0.004)                 | (-0.02–0.05)                  | (-0.02–0.05)            | (-0.06–0.02)                      |
|            |       | *p=0.50                        | *p=0.33                       | *p=0.48                 | *p=0.30                           |
|            |       | p=0.64                         | p=0.24                        | p=0.34                  | p=0.42                            |

Data are presented as effect sizes (95% CI) per allele and p-values.

**Effect sizes are adjusted for age, sex and BMI and are given as percentages since the traits are logarithmically transformed, and p-values are specified for the additive model and adjusted for age, sex and BMI. P-values without * are only adjusted for age and sex. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as (fasting plasma glucose (mmol/l)x fasting serum insulin (pmol/l))/22.5. Insulinogenic index was calculated as ((serum insulin 30 minutes post-oral glucose tolerance test (pmol/l) – fasting serum insulin (pmol/l)/plasma glucose 30 minutes post-oral glucose tolerance test (mmol/l)).

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newest approach in identifying BMI associated variants, the meta-analyses of ~125,000 individuals and independent replication in the same number, performed by the GIANT (Genomewide Investigation of ANThropometric measures) consortium, revealing 18 new BMI associated loci [42].

In conclusion, of the variants found to associate with obesity and related traits in the second GWAS wave, we were able to report association with obesity and/or measures of adiposity for variants in/near BDNF, TMEM18, ETV5, GNPDA2, SEC16B and FAIM2. Moreover, we found that rs4923461 in BDNF increase the risk of obesity, it conversely protect against type 2 diabetes, which could be through different neuronal and peripheral mechanisms.

Materials and Methods

Study populations

The 15 SNPs from the second obesity GWAS wave were genotyped in 18,014 individuals ascertained from four different study groups: 1) the Inter99 cohort, which is a population-based, randomised, non-pharmacological intervention study of middle-aged individuals for the prevention of ischaemic heart disease (n = 6,514), conducted at the Research Centre for Prevention and Health in Glostrup, Copenhagen (ClinicalTrials.gov ID-no: NCT00289237) [43]; 2) the ADDITION Denmark screening study cohort (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care) (ClinicalTrials.gov ID-no: NCT00237548) [44], which is a population-based, high-risk screening and intervention study for type 2 diabetes in general practice (n = 8,664); 3) a population-based group of unrelated middle-aged individuals (n = 680) examined at Steno Diabetes Center; and 4) unrelated type 2 diabetic patients (n = 2,158) sampled through the out-patient clinic at Steno Diabetes Center. In BMI stratified case-control analyses individuals from study group 2 with BMI<25 kg/m² were excluded. Hence, in the combined study sample 3,512 were normal weight, 7,458 were overweight, 5,044 were obese, and 340 were morbidly obese. Study groups 1 and 3 underwent a standard 75 g oral glucose tolerance test. Association with type 2 diabetes was evaluated in the combined study sample of which 5,302 were glucose-tolerant and 3,778 were type 2 diabetes patients. Definitions of overweight, obesity, morbid obesity and type 2 diabetes were according to WHO criteria, i.e. 25–29.99 kg/m², 30–39.99 kg/m² and ≥40 kg/m² respectively. In analyses of overweight individuals with BMI<25.0 kg/m² were compared with individuals in the BMI range 25–29.99 kg/m², obesity were analysed comparing BMI<25.0 kg/m² with the 30–39.99 kg/m² range, and finally analyses of morbid obesity were made with individuals with BMI<25.0 kg/m² and individuals with BMI≥40 kg/m².

Of note, a total of 5,586 individuals from study group 1 and 5,450 individuals from study group 2 were included in the GWAS performed by Thorleifsson et al. [19] (Note S1).

Statistical analyses

Case-control analyses were performed using logistic regression. Type 2 diabetes studies included the full study sample, whereas BMI stratified case-control analyses excluded individuals from study group 2 with BMI<25 kg/m² as controls, since this is a population of high-risk individuals. General linear models were used to test quantitative metabolic traits for differences between genotype groups in 6,039 treatment-naïve individuals from the population-based Inter99 cohort. All analyses were performed assuming an additive genetic model and with adjustments for age and sex. Additionally adjustments for BMI were introduced in case-control studies of type 2 diabetes and in quantitative trait analyses.

Quantitative traits that did not follow a normal distribution were logarithmically transformed. Statistical power in replication case-control settings was determined using the CaTS power calculator version 0.0.2. The lowest and the highest risk allele frequencies (RAF) of the examined SNPs were 8% and 84%, respectively, and the mean RAF was 50%. Using the population-based Inter99 cohort as reference, the prevalence of overweight, obesity, morbid obesity and type 2 diabetes in the Danish population was estimated to 39%, 17%, 1.3% and 8%, respectively. Estimated statistical power calculations in case-control settings are presented in Table S2.
Statistical power for the quantitative traits was estimated using
simulations ($n = 5,000$), where variance across genotypes was
drawn from phenotypes simulated to follow normal distribution
using empirical variances. The variance for adjustment factors,
estimated using residuals of linear models, was also included in the
model, assuming independence of genotypes. Linear models were
used both for simulating and testing data, assuming additive
models and using a significance threshold of 0.05. The estimated
statistical power for variants with a RAF of 8%, 94% and 50%
respectively, were determined and are presented in Table S3. All
analyses were performed in RGui version 2.8.0. Due to the large
amount of tests performed in the present study, we used
Bonferroni correction for multiple testing. For the primary
hypothesis traits this resulted in a significance level of $p < 0.0004$,
wheras it for follow-up traits resulted in a significance level of
$p < 0.0001$.

Supporting Information

Table S1 Clinical characteristics of the study groups comprising the combined study population.

Table S2 Statistical power calculations in replication case-control settings.

Table S3 Statistical power calculations of quantitative traits.

Table S4 Case-control studies of overweight, obesity and type 2 diabetes in the combined study sample.

Table S5 Anthropometric and metabolic measures in the population-based Inter99 study sample.

Note S1 Individuals included from the Inter99 cohort.

Note S2 SNPs selected for genotyping.

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Author Contributions

Conceived and designed the experiments: CHS MAV KA TJ TH OP. Performed the experiments: CHS MAV DS. Analyzed the data: CHS MAV. Contributed reagents/materials/analysis tools: LH AS TL DW TJ OP. Wrote the paper: CHS MAV DSB.
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