Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 772–777
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Effects of genetic variation in the visfatin gene (*PBEF1*) on obesity, glucose metabolism, and blood pressure in children

Antje Körner[a], Yvonne Böttcher[b], Beate Enigk[b], Wieland Kiess[a], Michael Stumvoll[b], Peter Kovacs[b,*]

[a]*University Hospital for Children and Adolescents, University of Leipzig, D-04103 Leipzig, Germany*
[b]*Medical Department III, University of Leipzig, D-04103 Leipzig, Germany*

Received 12 October 2006; accepted 4 January 2007

Abstract

Visfatin is a peptide predominantly expressed in visceral adipose tissue and was hypothesized to be related to obesity and insulin resistance. In the present study, we investigated the effects of genetic variations in the visfatin gene (pre-B-cell colony–enhancing factor 1 [*PBEF1*]) on obesity, metabolic parameters, and blood pressure (BP) in 2 children cohorts. We genotyped 3 representative single nucleotide polymorphisms (rs9770242, −948G>T, rs4730153) in 731 schoolchildren and in an independent cohort of 167 obese children. There was no association of any of the 3 polymorphisms or their haplotypes with body mass index (BMI), waist-to-hip ratio, or parameters of glucose, insulin, or lipid metabolism in either cohort. However, the −948G variant was associated with significantly higher diastolic BP in obese children ($P < .05$ after adjusting for age, sex, pubertal stage, and height). Haplotype analyses confirmed the [T-G-A] haplotype to be significantly related to increased diastolic BP in both schoolchildren and obese children. In conclusion, genetic variants in *PBEF1* may be associated with *increased BP* in children but are not likely to contribute significantly to the variation in body mass index and glucose, insulin, or lipid metabolism.

1. Introduction

Visceral obesity is associated with an increased prevalence of insulin resistance, type 2 diabetes mellitus, and cardiovascular disease [1]. This particular association of comorbidities with a visceral pattern of body fat distribution is related to differences in gene expression and intrinsic fat depot–specific differences in the endocrine function of adipose tissue [2]. Visfatin, by application of a differential display method, was identified as a peptide predominantly expressed and secreted from visceral adipose tissue in humans and mice [3]. Visfatin plasma concentrations correlate positively with the messenger RNA expression in visceral fat, and both are related to body mass index (BMI) and total percentage of body fat [4]. Interestingly, visfatin plasma levels are increased in morbid obesity and type 2 diabetes mellitus [5,6]. These findings indicate that visfatin may play a role in the association between visceral obesity and increased metabolic risk [7], and the visfatin gene (pre-B-cell colony–enhancing factor 1 [*PBEF1*]) may, hence, constitute a candidate gene involved in susceptibility to the metabolic syndrome. Recent studies on the *PBEF1* gene from our laboratory suggested that genetic variation in the visfatin gene may, indeed, have a minor effect on visceral and subcutaneous visfatin messenger RNA expression profiles and parameters of glucose and insulin metabolism [8].

Environmental factors strongly modify the phenotype on a given genetic predisposition and may even mask the effects of genes on complex phenotypes. Considering this interaction, children represent a particularly interesting study population because their phenotype is less influenced by confounding factors (comorbidities, treatment, etc) compared with that in adults. Thus, relevant gene polymorphisms should affect the phenotypes at a young age, and gene-phenotype relationship may emerge more clearly. The aim of the present study was, therefore, to evaluate the

* Corresponding author. Tel.: +49 341 97 15892; fax: +49 341 9713389.
E-mail address: peter.kovacs@medizin.uni-leipzig.de (P. Kovacs).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.01.009

effects of *PBEF1* genetic variants on obesity and parameters of the metabolic syndrome in representative cohorts of white schoolchildren and obese children.

2. Materials and methods

2.1. Subjects

2.1.1. Healthy lean children

Healthy children were selected from the Leipzig Schoolchildren Project that investigated anthropometric and clinical parameters in 2500 children and adolescents aged 6 to 17 years from 1999 to 2001 [9] and constitutes a representative healthy population of white children with normal distribution of BMI. DNA was available in 731 schoolchildren (355 boys, 376 girls). Of this cohort, 508 children with a BMI of between −1.0 and 1.0 SD score (SDS) (243 boys, 265 girls; mean age, 12.0 ± 0.12 years) were selected to serve as the healthy normal-weight control group.

2.1.2. Obesity cohort

A total of 167 white children and adolescents (89 boys, 78 girls; 11.6 ± 0.25 years; BMI-SDS, 2.71 ± 0.04) were consecutively recruited from the obesity clinic of the University Hospital for Children & Adolescents (Leipzig, Germany). All obese children had a detailed metabolic work-up including an oral glucose tolerance test and lipid profile.

In all subjects, a careful history and physical examination including anthropometric measurements were obtained. Height and weight were determined using precision stadiometers and scales to the nearest 0.1 cm and 0.1 kg, respectively. For standardization of height, weight, and BMI, reference percentiles for central Germany were applied [10], and data are given as absolute values and/or SDSs.

Written informed consent had been obtained from the patients' guardians, and the study was approved by the ethical committee of the University of Leipzig.

2.2. Genotyping of visfatin single nucleotide polymorphisms

Genotyping of the polymorphisms was performed by the TaqMan allelic discrimination assay (Custom TaqMan SNP Genotyping Assay; Applied Biosystems, Foster City, CA). Oligonucleotide sequences were as follows: rs9770242, forward—5′-CCAACTCGTTTCCCAGGATTTAAAG-3′, reverse— 5′-ACGGGCCAAGCCTTTGA-3′, reporter [VIC/FAM]—CAGT[G/T]TCGCACCCTG; −948G>T, forward—5′-GCCCGTTGCCTTTTCCTT-3′, reverse—5′-GGTGGAATTCAGTCCTCACAGATAA-3′, reporter [VIC/FAM]—CCTAATTGAAC[C/A]GAGTATT. Genotyping of rs4730153 was done using Assays-on-Demand, SNP Genotyping Products (C_2673294_10; Applied Biosystems). The reaction was amplified on a GeneAmp polymerase chain reaction system 9700 (95°C for 10 minutes, 95°C for 15 seconds, 62°C for 1 minute, for 38 cycles; Applied Biosystems), and fluorescence was detected on an ABI PRISM 7700 sequence detector (Applied Biosystems). A random ~10% selection of the sample was re-genotyped in both single nucleotide polymorphisms (SNPs); all genotypes matched the initial designated genotypes.

2.3. Statistical analyses

Before statistical analysis, nonnormally distributed parameters were logarithmically transformed to approximate a normal distribution. Differences in genotype frequencies were compared using logistic regression between the obese and healthy controls. Multivariate linear relationships were assessed by a generalized linear model. In the additive model, homozygotes for the major allele (MM), heterozygotes (Mm), and homozygotes for the minor allele (mm) were coded to a continuous numeric variable for genotype (as 0, 1, 2). A dominant model was defined as contrasting genotypic group MM + Mm vs mm, and the recessive model was defined as contrasting genotypic group MM vs Mm + mm. In haplotype analyses, groups of subjects carrying 2, 1, or 0 copy of the haplotype were compared.

Statistical analyses were performed using the SPSS software package (version 11.5) (SPSS, Chicago, IL) and the statistical analysis system of the SAS Institute (Cary,

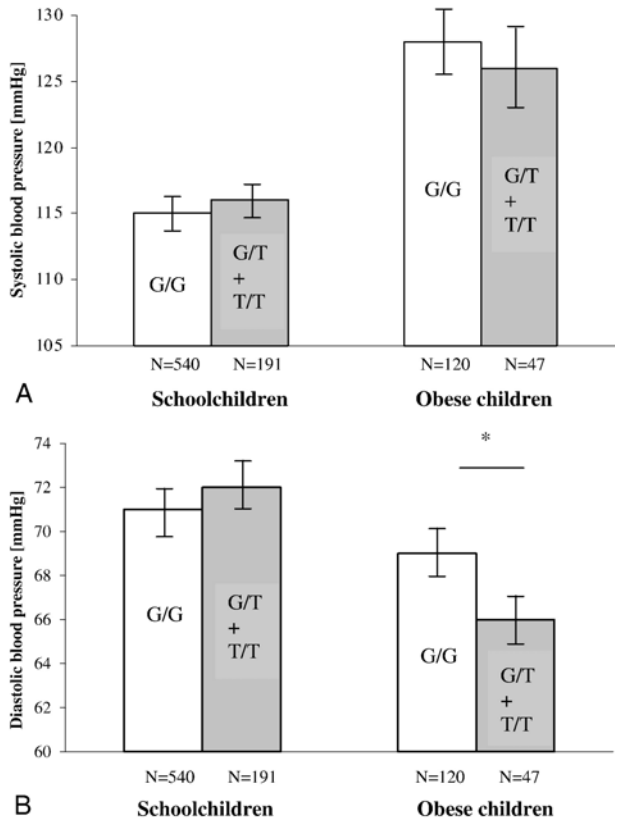

Fig. 1. Effect of the −948G>T *PBEF1* variant on systolic and diastolic BP in schoolchildren and obese German children. Because of a small sample size of the T/T group, G/T and T/T subjects were combined for statistical analyses. *$P < .05$ after adjusting for age, sex, pubertal stage, height, and BMI. G/G indicates subjects homozygous for the G allele; G/T, heterozygotes; T/T, homozygotes for the T allele.

Table 1
Clinical characteristics of German obese children grouped by *PBEF1* haplotypes

Haplotype	[T-G-G]			[T-G-A]			[G-G-A]			[G-T-A]		
	2 Copies	1 Copy	0 Copy	2 Copies	1 Copy	0 Copy	2 Copies	1 Copy	0 Copy	2 Copies	1 Copy	0 Copy
Obese children	n = 59	n = 82	n = 26	n = 3	n = 47	n = 117	n = 2	n = 32	n = 133	n = 1	n = 42	n = 124
Sex (M/F)	29/30	37/45	12/14	2/1	18/29	58/59	1/1	12/20	65/68	1/0	22/20	55/69
Age (y)	12 ± 0.4	12 ± 0.4	12 ± 0.7	10 ± 2.6	11 ± 0.5	12 ± 0.3	13 ± 1.5	13 ± 0.6	11 ± 0.3	14 ± NA	12 ± 0.6	12 ± 0.3
BMI (kg/m^2)	31 ± 1	31 ± 1	31 ± 1	30 ± 1	31 ± 1	31 ± 1	31 ± 3	31 ± 1	31 ± 1	32 ± NA	30 ± 1	31 ± 1
BMI (SDS)	2.73 ± 0.07	2.67 ± 0.06	2.75 ± 0.09	2.94 ± 0.47	2.75 ± 0.08	2.68 ± 0.05	2.53 ± 0.28	2.67 ± 0.09	2.71 ± 0.05	2.47 ± NA	2.67 ± 0.08	2.71 ± 0.05
WHR	0.88 ± 0.01	0.88 ± 0.01	0.87 ± 0.01	0.90 ± 0.04	0.88 ± 0.01	0.88 ± 0.01	0.88 ± 0.03	0.88 ± 0.02	0.88 ± 0.01	0.91 ± NA	0.88 ± 0.01	0.88 ± 0.01
Fasting plasma glucose (mmol/L)	4.76 ± 0.06	4.68 ± 0.05	4.77 ± 0.08	4.57 ± 0.14	4.76 ± 0.06	4.71 ± 0.04	4.54 ± 0.12	4.80 ± 0.08	4.70 ± 0.04	5.20 ± NA	4.64 ± 0.07	4.74 ± 0.04
2-h plasma glucose (mmol/L)	6.10 ± 0.11	6.04 ± 0.10	6.20 ± 0.19	6.41 ± 0.38	6.05 ± 0.12	6.09 ± 0.09	7.54 ± 2.19	6.07 ± 0.14	6.07 ± 0.08	5.81 ± NA	6.08 ± 0.14	6.09 ± 0.08
Fasting plasma insulin (pmol/L)	92 ± 6	84 ± 6	95 ± 11	96 ± 25	85 ± 7	90 ± 5	80 ± 8	82 ± 8	90 ± 5	190 ± NA	93 ± 10	86 ± 4
HOMA	2.75 ± 0.21	2.47 ± 0.17	2.78 ± 0.37	2.76 ± 0.78	2.49 ± 0.22	2.67 ± 0.15	2.26 ± 0.27	2.38 ± 0.26	2.68 ± 0.14	6.12 ± NA	2.72 ± 0.30	2.55 ± 0.13
TG	1.34 ± 0.09	1.27 ± 0.07	1.20 ± 0.10	1.46 ± 0.26	1.14 ± 0.09	1.34 ± 0.06	1.55 ± 0.05	1.26 ± 0.11	1.29 ± 0.06	1.55 ± NA	1.30 ± 0.10	1.28 ± 0.06
Total cholesterol	4.19 ± 0.11	4.23 ± 0.09	4.01 ± 0.19	4.64 ± 0.28	4.06 ± 0.12	4.22 ± 0.08	4.71 ± 0.11	4.13 ± 0.13	4.18 ± 0.08	2.97 ± NA	4.17 ± 0.17	4.19 ± 0.07
HDL cholesterol	1.22 ± 0.04	1.24 ± 0.03	1.19 ± 0.06	1.34 ± 0.03	1.23 ± 0.04	1.22 ± 0.03	0.99 ± 0.29	1.23 ± 0.06	1.22 ± 0.02	0.82 ± NA	1.21 ± 0.04	1.23 ± 0.03
LDL cholesterol	2.59 ± 0.09	2.58 ± 0.08	2.57 ± 0.11	2.96 ± 0.14	2.53 ± 0.08	2.60 ± 0.07	2.97 ± 0.04	2.47 ± 0.11	2.60 ± 0.06	1.67 ± NA	2.65 ± 0.13	2.57 ± 0.06
LDL/HDL ratio	2.24 ± 0.11	2.18 ± 0.08	2.24 ± 0.15	2.22 ± 0.11	2.13 ± 0.10	2.25 ± 0.08	3.30 ± 0.99	2.13 ± 0.14	2.21 ± 0.07	2.04 ± NA	2.24 ± 0.12	2.20 ± 0.07
Systolic BP (mm Hg)	126 ± 2	129 ± 2	127 ± 5	136 ± 8	128 ± 3	128 ± 2	132 ± 0	131 ± 4	127 ± 1	131 ± NA	126 ± 3	128 ± 2
Diastolic BP (mm Hg)	69 ± 1	68 ± 1	70 ± 2	81 ± 2	70 ± 2	68 ± 1*	70 ± 6	69 ± 3	68 ± 1	62 ± NA	66 ± 1	70 ± 1*

The data are presented as mean ± SEM. *$P < .05$ in an additive mode of inheritance; P values were calculated after adjusting for age, sex, and BMI. Haplotypes are defined by the composition of alleles at each SNP in the following order: [rs9770242]-[−948G>T]-[rs4730153]. HOMA indicates homeostasis model assessment; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable.

NC). P values of less than .05 were considered to be statistically significant.

3. Results

3.1. Genetic variation in the visfatin gene

Three representative SNPs (−1001T>G [rs9770242], −948G>T, intron 6 c.744 −87G>A [rs4730153]), recently identified by sequencing the entire visfatin gene [8], were genotyped in 731 German schoolchildren and 167 obese children. Minor allele frequencies in the representative schoolchildren cohort were as follows: G = 0.25 for (rs9770242), T = 0.14 for −948G>T, and A = 0.42 for (rs4730153). Genotypic distribution of all variants was in Hardy-Weinberg equilibrium (all $P > .10$). Although all SNPs were in high linkage disequilibrium (D' = 0.98-0.99), their r^2 value was relatively low (.23-.49), which necessitates genotyping of all 3 variants for association analyses. In addition, by computational analysis [11,12], we identified 4 common haplotypes (frequency of each haplotype >0.05) among the 3 SNPs in our cohort that accounted for more than 98% of all observed haplotypes: haplotype [T-G-A] (14%), [T-G-G] (61%), [G-G-A] (10%), and [G-T-A] (14%), where haplotypes are defined by the composition of alleles at each SNP in the following order: [rs9770242]-[−948G>T]-[rs4730153].

3.2. Association studies in schoolchildren

There was no association of the visfatin SNPs with BMI and BMI-SDS in the schoolchildren (n = 731), whereas there were mild differences in the waist-to-hip ratio (WHR). Children homozygous for the T allele of the rs9770242 SNP (T/T) had a higher mean WHR than T/G heterozygotes and G/G homozygotes (0.81 ± 0.003 vs 0.80 ± 0.003 vs 0.79 ± 0.008; $P < .05$ after adjusting for age and sex). Haplotype analyses revealed a significantly higher diastolic blood pressure (BP) of the [T-G-A] haplotype carriers compared with subjects without the haplotype (72 ± 0.6 vs 71 ± 0.3; $P < .01$ after adjusting for age, sex, pubertal stage, height and BMI). Conversely, the [G-G-A] haplotype was significantly associated with lower diastolic BP (70 ± 0.6 vs 71 ± 0.3; $P < .05$ after adjusting for age, sex, pubertal stage, height, and BMI). There was no association of any of the haplotypes with BMI, BMI-SDS, WHR, or systolic BP (data not shown).

3.3. Association studies in obese children

There were no statistically significant differences in genotype and haplotype frequency or distribution between obese children (n = 167) and the lean control group (n = 508) in any of the examined polymorphisms (data not shown).

Similar to the schoolchildren cohort, there was no association of the SNPs with BMI, BMI-SDS, or WHR. There was also no association of genotypes with parameters of glucose metabolism (fasting and 2-hour plasma glucose, fasting plasma insulin, homeostasis model assessment of insulin resistance) or lipid phenotypes (serum total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, triglycerides) (all $P > .05$ after adjusting for age, sex, and BMI; data not shown).

However, as observed in the normal cohort, we identified an association of the SNPs with diastolic BP that was conferred by the −948G allele with a significant association with increased diastolic BP in a recessive mode of inheritance (Fig. 1). This effect of the −948G>T polymorphism on diastolic BP was also reflected in the haplotype analyses with a significantly higher diastolic BP in obese children with the [T-G-A] haplotype and a lower diastolic BP in the [G-T-A] haplotype carriers (Table 1).

4. Discussion

Visfatin is a newly identified adipocytokine with predominant expression in the visceral fat depot that exerts insulin-like actions on glucose metabolism through direct interaction with the insulin receptor [7,13]. The plasma levels of visfatin are closely related to body fat content and are increased in morbid obesity and type 2 diabetes mellitus [5,6]. The human visfatin (*PBEF1*) maps to a region on chromosome 7q22.3 [14] previously found to be related to the metabolic syndrome in nondiabetic Mexican Americans [15] and to BMI in a combined analysis of genomewide linkage scans for BMI [16]. In addition, this region overlaps with the position of a coincident linkage to fasting plasma insulin and BP in hypertensive Hispanic families in a multipoint variance component analysis [17]. Hence, variation in the visfatin gene may contribute to the genetic predisposition for metabolic syndrome and its underlying pathophysiologic mechanisms.

In the present study, we aimed to assess the effects of previously identified *PBEF1* genetic variants on insulin, glucose, and lipid metabolism, BP, and obesity in cohorts of schoolchildren and obese children from Germany. The children cohorts were chosen to reduce the effects of potential environmental components on contribution of visfatin genetic polymorphisms to variation of traits related to the metabolic syndrome. In contrast to previous observations of visfatin genetic variants with parameters of glucose metabolism in adult whites [8], there was no correlation of lipid phenotypes, or glucose or insulin metabolism with any of the 3 examined polymorphisms in either representative schoolchildren or obese children. Whether the effects of genetic variants on glucose and insulin metabolism observed in adults are due to interaction with environmental factors to which children might be less exposed remains to be clarified.

Nevertheless, variants in the *PBEF1* gene were significantly associated with diastolic BP, particularly in obese children. Although the differences in the mean diastolic BP were small and may thus be regarded as coincidental, haplotype analyses confirmed an association of the [T-G-A]

haplotype with increased diastolic BP in both healthy school children and obese children that was, again, more profound in obese children. Although 3 of 4 haplotypes were significantly correlated with diastolic BP, this simply seems to reflect phenotypic effects of the −948G>T. However, we are also aware that this SNP may not be a functional variant within the visfatin gene, but is in high linkage disequilibrium with a nearby functional variant. Although a prevalent coincidence of hypertension and insulin resistance might partially explain the link between visfatin and the *increase in BP*, there was no relationship between the clinical parameters of insulin metabolism and variation in the visfatin gene in the present study. Therefore, it is likely that mechanisms other than insulin-related pathways are responsible for the observed association between visfatin genetic variants and BP. There is evidence that obesity-related sequelae such as cardiovascular alterations and increased BP occur early in life and already in childhood, and that adipocytokines may play a role in this association [18]. In a study on young obese subjects from Italy, diastolic BP correlated directly with WHR and plasma renin activity in both normotensive and hypertensive obese subjects and with fasting serum insulin in normotensive obese subjects [19]. Multiple regression analysis indicated that diastolic BP values increased with WHR, fasting serum insulin, and plasma renin activity, but not with BMI, urinary sodium excretion, and norepinephrine levels, indicating that increased plasma renin activity plays an important role in the development of hypertension in subjects with central obesity [19]. Studies on the relation of visfatin genetic variants with plasma renin activity might therefore be of potential interest in pinpointing the mechanisms underlying genetic associations with diastolic BP.

We are aware that we have not statistically allowed for the number of comparisons made and the results need to be interpreted with caution. However, to do so would raise the threshold for significant results and might lead to discarding some findings that could be meaningful. According to the published point of view of Kenneth J. Rothman, Editor of Epidemiology, who explains in his editorial why he thinks that "No adjustments are needed for multiple comparisons," while remaining alert to the problem, the unadjusted and as such declared *P* values should be given and the data exposed to verification by others [20]. Replication of initial findings seems to be the most appropriate way to overcome the problem of multiple testing. In line with this, in our study we provide significant associations of the visfatin [T-G-A] haplotype with increased diastolic BP in 2 independent cohorts.

In conclusion, in children, genetic variants in the *PBEF1* are not likely to contribute significantly to the variation in BMI and glucose, insulin, or lipid metabolism. However, based on our present association with diastolic BP in 2 independent children cohorts, genetic variants in the visfatin gene are potential candidates for explaining the linkage of 7q22.3 with BP and traits of the metabolic syndrome. It remains to be clarified whether the lack of association of genetic variants with glucose and insulin metabolism in children is because of missing interaction with environmental factors to which children might be less exposed compared with adults.

Acknowledgments

This work was supported by grants from the Deutsche Forschungsgemeinschaft (KFO-152 "Atherobesity" to AK [KO3512/1-1], WK [BE1264/10-1], and MS), from the German Diabetes Association (to AK and PK), from the European Commission PIONEER (to WK), from the Interdisciplinary Center for Clinical Research at the University of Leipzig (Z14 and B27 to MS, PK, and BE), and from the German Hypertension Association (to AK). The Leipzig Schoolchildren Project was supported by unrestricted educational grants from Pfizer Pharma and Novo Nordisk to WK.

We thank all the children who participated in the studies. We appreciate the help of the nurses and physicians who performed the clinical examinations and data collection.

References

[1] Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000;21:697-738.
[2] Blüher M, Patti ME, Gesta S, et al. Intrinsic heterogeneity in adipose tissue of fat-specific insulin receptor knock-out mice is associated with differences in patterns of gene expression. J Biol Chem 2004;279:31891-901.
[3] Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005;307:426-30.
[4] Berndt J, Kloting N, Kralisch S, et al. Plasma visfatin concentrations and fat depot–specific mRNA expression in humans. Diabetes 2005;54:2911-6.
[5] Haider DG, Schindler K, Schaller G, et al. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. J Clin Endocrinol Metab 2006;91:1578-81.
[6] Chen MP, Chung FM, Chang DM, et al. Elevated plasma level of visfatin/pre-B cell colony–enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2006;91:295-9.
[7] Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin—the classical, resistin—the controversial, adiponectin—the promising, and more to come. Best Pract Res Clin Endocrinol Metab 2005;19:525-46.
[8] Böttcher Y, Teupser D, Enigk B, et al. Genetic variation in the visfatin gene (*PBEF1*) and its relation to glucose metabolism and fat depot specific mRNA expression in humans. J Clin Endocrinol Metab 2006;91:2725-31.
[9] Reich A, Müller G, Gelbrich G, et al. Obesity and blood pressure—results from the examination of 2365 schoolchildren in Germany. Int J Obes 2003;27:1459-64.
[10] Kromeyer-Hauschild K, Wabitsch M, Geller F, et al. Perzentile für den Body Mass Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. Monatsschr Kinderheilkd 2001;149:807-18.
[11] Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162-9.
[12] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978-89.

[13] Hug C, Lodish HF. Medicine. Visfatin: a new adipokine. Science 2005;307:366-7.
[14] Ognjanovic S, Bao S, Yamamoto SY, et al. Genomic organization of the gene coding for human pre–B-cell colony enhancing factor and expression in human fetal membranes. J Mol Endocrinol 2001;26:107-17.
[15] Arya R, Blangero J, Williams K, et al. Factors of insulin resistance syndrome-related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic Mexican-Americans. Diabetes 2002;51:841-7.
[16] Wu X, Cooper RS, Borecki I, et al. A combined analysis of genomewide linkage scans for body mass index from the National Heart, Lung, and Blood Institute Family Blood Pressure Program. Am J Hum Genet 2002;70:1247-56.
[17] Cheng LS, Davis RC, Raffel LJ, et al. Coincident linkage of fasting plasma insulin and blood pressure to chromosome 7q in hypertensive Hispanic families. Circulation 2001;104: 1255-60.
[18] Körner A, Kratzsch J, Gausche R, Kiess W. New predictors of the metabolic syndrome in children—role of adipocytokines? Pediatr Res 2007 [in press].
[19] Licata G, Scaglione R, Ganguzza A, et al. Central obesity and hypertension. Relationship between fasting serum insulin, plasma renin activity, and diastolic blood pressure in young obese subjects. Am J Hypertens 1994;7:314-20.
[20] Rothman KJ. "No adjustments are needed for multiple comparisons". Epidemiology 1990;1:1.