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Brief report

Genome-wide search for susceptibility genes to type 2 diabetes in West Africans: Potential role of C-peptide

Guanjie Chen [a,*], Adebowale Adeyemo [a], Jie Zhou [a], Yuanxiu Chen [a], Hanxia Huang [a], Ayo Doumatey [a], Kerrie Lashley [a], Kofi Agyenim-Boateng [b], Benjamin A. Eghan Jr. [b], Joseph Acheampong [b], Olufemi Fasanmade [c], Thomas Johnson [c], Godfrey Okafor [d], Johnnie Oli [d], Albert Amoah [e], Charles Rotimi [a]

[a] *National Human Genome Center at Howard University, College of Medicine, Washington, DC, United States*
[b] *University of Science and Technology, Department of Medicine, Kumasi, Ghana*
[c] *University of Lagos, College of Medicine, Endocrine and Metabolic Unit, Lagos, Nigeria*
[d] *University of Nigeria Teaching Hospital, Department of Medicine, Enugu, Nigeria*
[e] *University of Ghana Medical School, Department of Medicine, Accra, Ghana*

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Abstract

C-peptide is a substance that the pancreas releases into the circulation in equimolar amounts to insulin and has demonstrated important physiological effects which relate to the vascular field, in particular the microcirculation. For this analysis, we included 321 full and 36 half sibling pairs affected with type 2 diabetes (T2D) from West Africa. A genome-wide panel of 390 tri-nucleotide and tetra-nucleotide repeats with an average distance of 8.9 cM was performed on a total of 691 persons. Variance components based on multipoint linkage approach as implemented in SOLAR were performed for log C-peptide. Significant linkage evidences were observed on 10q23 at D10S2327 with a LOD score of 4.04 (nominal p-value = 0.000008, empirical p-value = 0.0004); and on 4p15 at D4S2632 with a LOD score of 3.48 (nominal p-value = 0.000031, empirical p-value = 0.0013). Other suggestive evidence of linkage were observed on 15q14 at D15S659 with a LOD score 2.41 (nominal p-value = 0.000435, empirical p-value = 0.0068), and on 18p11 near D18S976 with a LOD score 2.18 (nominal p-value = 0.000771 and empirical p-value = 0.0094). Interestingly, five positional candidate genes for diabetes and related complications are located in our linkage region (the pituitary adenylate cyclase activating polypeptide (PACAP in 18p11); the peroxisome proliferator-activated receptor gamma coactivator 1 (PPARGC1 in 4p15); PTEN, PPP1R5, and IDE located in 10q23. In conclusion, we identified four major genetic loci (10q23, 4p15, 15q14, and 18p11) influencing C-peptide concentration in West Africans with T2D.

Keywords: C-peptide; Genome scan; Type 2 diabetes; West Africa

1. Introduction

C-peptide is secreted into the bloodstream by the pancreas along with insulin. Since half-life of C-peptide in plasma is longer than insulin, C-peptide concentration is 5–10 times higher than that of insulin. The main

* Corresponding author at: National Human Genome Center, Howard University, Genetic Epidemiology Unit, College of Medicine, 2216 6th Street, NW, Washington, DC 20059, United States.
Tel.: +1 202 806 9437; fax: +1 202 265 2254.
E-mail address: gchen@howard.edu (G. Chen).

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physiological role of C-peptide is to facilitate the folding of the pro-insulin molecule. However, recent studies have been demonstrated physiological effects of C-peptide involving renal and nerve function, and the stimulation of whole body glucose uptake in patients with type 1 diabetes [1–4]. For example, a positive linear relationship has been observed between residual plasma C-peptide concentration and erythrocyte Na^+–K^+-ATPase activity in a cohort of patients with T2D [5]. Under diabetic conditions, Na^+, K^+-ATPase activity is decreased in different cell types and might be involved in the pathogenesis of various diabetic complications [6–8]. In contrast, similar physiologic effects of C-peptide have not been observed in health subjects or animals suggesting a saturation of the mechanisms of C-peptide action in health subjects [6]. These growing physiologic importances of C-peptide prompted us to considerate C-peptide as a phenotype in whole-genome linkage studies for T2D.

2. Research design and methods

2.1. Subjects

Participants included in these analyses were enrolled in the AADM study as described in detail by Rotimi et al. [9,10]. The protocol was approved by Institutional Review Board (IRB) of each institution and written informed consent was obtained from all participants. At the time of these analyses, the AADM study enrolled only affected pairs with T2D. In this regard, data on parents and other family members were not available. Participants were enrolled from three centers in Nigeria (Enugu, Ibadan and Lagos) and two centers in Ghana (Kumasi and Accra). Diabetes diagnosis was based on the criteria established by the America Diabetes Association Expert Committee as follows: a fasting plasma glucose (FPG) concentration $>$ 126 mg/dl (7.0 mmol/l) or a 2-h post-load value in the OGTT $>$ 200 mg/dl (11.1 mmol/l) on more than one occasion. The detection of auto-antibodies to glutamic acid decarboxylase (GAD) antibody and/or a fasting C-peptide $<$ 0.03 nmol/l were used to exclude probable cases of type 1 diabetes. Probands were required to: (1) be older than 25 years at the time of diagnosis of T2DM; (2) have at least one full sibling with T2DM; (3) not have classical features of type 1 diabetes (i.e., insulin-dependence, repeated episodes of ketoacidosis); and (4) not have cases of type 1 diabetes in first degree relatives. The diagnosis of diabetes was based on the 1999 America Diabetes Association criteria.

2.2. Measurements

Detailed clinic examination was conducted on each participant including physical exam, medical history, anthropometric measurements, blood pressure and venous puncture to obtain blood samples for biochemistries and genetic analyses. A minimum of 8-h fasting blood samples were obtained from all participants for the assessment of multiple metabolic traits including glucose, insulin, C-peptide, basic chemistries and complete lipid profile in centralized reference labs in the Northwest Lipid Research Laboratories and the Diabetes Endocrinology Research Center (DERC) immunoassay Core Laboratory in Seattle, Washington. In brief, the C-peptide radioimmunoassay uses a commercially available antibody from Linco and the assay is a 48-h PEG accelerated assay. The antibody is produced in guinea pigs by repeated immunization with highly purified human C-peptide.

2.3. Genotyping

Genotyping was carried out at the Center for Inherited Disease Research (CIDR). The CIDR marker set is composed primarily of tri-nucleotide and tetra-nucleotide repeats and consists of 392 primer pairs with average distance of 8.9 cM throughout the genome. The average marker heterozygosity is 0.76. For AADM study, 390 short tandem repeat markers were genotyped for an average sex-equal distance of 8.9 cM and with no gaps greater than 18 cM. Extensive quality checks were carried out to verify consistency of marker genotyping and stated pedigree relations. We used all 390 markers to check pedigree errors by S.A.G.E.-REALTEST [11] which is based on likelihood method to check the degree of biological relationship for each sibpair and as a result, 36 sibling pairs were re-assigned as half sibling pairs according to the results of REALTEST procedure. Then, PedCheck [12] was used to check for Mendelian inconsistency. Inconsistency rate was 0.11%. Errors identified in PedCheck were assumed to have occurred in the genotyping process and the associated mar-kers were set to missing among the appropriate pedigree. Hardy–Weinberg equilibrium at each locus was assessed by the χ^2 test [13].

2.4. Statistical analysis

Descriptive statistics were calculated using SAS (The SAS Institute, Cary, NC, USA). Covariates were included in linkage analyses if they explained a significant portion of the variation (significant parameter estimate $p \leq 0.05$ and/or increase in model $r^2 \geq 0.01$) in the phenotype. The covariate meeting these criteria that were adjusted for are duration of T2D, treatment of insulin, insulin, and gender. We did not adjust for oral hypoglycemic agents because over 80% of the subjects were on oral agents for blood glucose control. We performed a logarithmic transformation to improve the skewness and kurtosis of the data [14]. Heritability of log C-peptide was estimated by means of variance component modeling as implemented in SOLAR software package [15]. Covariates in the model were duration of T2D, treated with insulin (yes versus no), insulin, and gender. QTL linkage analysis of log C-peptide was performed using the multipoint variance component approach in SOLAR software package [15]. We used the log C-peptide in all linkage analyses because of the sensitivity of the variance components method to assumptions of normal-

ity. In variance components linkage analysis, the variance of a trait is decomposed into locus-specific effects determined by the identity-by-descent (IBD) relationships (additive QTL variance), the residual additive genetic effects (additive polygenic variance) and individual specific random environmental effects (random environmental variance).

The null hypothesis is that the additive QTL variance equals zero (no linkage) and this was tested by comparing the likelihood of the restricted model with that of a model in which the additive QTL variance is estimated. The difference between the two log likelihoods produces a logarithm of odds (LOD) score. Twice the difference between the two log likelihoods of these models yields a test statistic that is asymptotically distributed as a 50:50 mixture of a x^2 variable and a point mass at zero. SOLAR assessed linkage by fitting a polygenic model which does not incorporate genetic marker information and comparing it with model which incorporate genotype data at a specific marker (two point analyses) or across a chromosome (multipoint analysis). The null hypothesis of no linkage at a specific chromosomal location was tested by comparison of a polygenic model to a model with genetic variance components for both a QTL and polygenic factors. The log(base 10) of the ratio of the likelihoods of the polygenic and maker-specific models is the log of the odds score (LOD). A multivariate normal distribution is assumed in SOLAR. The significant level for covariates was p-value < 0.05.

Marker map order and positions were taken as that on sex-averaged genetic maps provided by the Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics/Map_markers/maps). A LOD score ≥ 3.3 is considered as strong evidence of linkage and a LOD score ≥ 1.9 but <3.3 as suggestive evidence of linkage [16].

3. Results

Clinical characteristics of the affected subjects are shown in Table 1. The study sample included 691 persons (59.3% women) with T2D. The mean age was 53 years, age at diagnosis was 48 years and mean duration of diabetes at the time of the study was 7 years.

Table 1
Demographic characteristics of study participants

	Male	Female
No. of individuals (%)	281 (40.67)	410 (59.33)
Families (n)	343	
Sibpairs (n)	321	
Half sibpairs (n)	36	
Age (years)	53.8 ± 10.5	53.0 ± 11.1
Height (cm)**	169.6 ± 7.2	159.0 ± 7.00
Body mass index (kg/m^2)**	25.0 ± 5.3	27.3 ± 5.6
C-peptide (ng/ml)**	1.09 ± 0.7	1.36 ± 0.78
Fasting insulin (μU/ml)	19.6 ± 27.0	23.2 ± 32.0
Fasting glucose (mg/dl)	198.5 ± 95.6	202.8 ± 86.8

Data are means ± S.D. unless otherwise indicated for diabetes individuals. Represents significant difference comparing with women **($p < 0.01$).

Mean value of C-peptide, insulin and glucose were 1.25 ng/ml, 21.70 μU/ml, and 201.00 mg/dl, respectively. The correlation between the log C-peptide value and covariates are shown in Table 2. The maximum likelihood heritability estimates for log C-peptide was 0.72 (S.E. 0.095, $p = 5.42 \times 10^{-12}$). Most of the subjects (79%) were on oral hypoglycemic agents (OHA) only, 8% were on insulin, 2.5% were on both insulin and OHA, and 8% were on dietary management only. Approximately 50% of these diabetes patients were also hypertensive.

Results of the multipoint linkage analysis for log C-peptide with duration of T2D, treated with insulin, insulin and gender as covariates are displayed in Figs. 1 and 2 and a summary of the results is displayed in Table 3. Two significant linkage regions and two suggestive linkage regions were identified. Ten thousand simulations were performed to determine the robust corrected LOD score and corresponding empirical probability values for peaks observed on chromosome 4, 10, 15, and 18, respectively. The strongest evidence was located on 10q23 region near D10S2327 with a LOD score 4.04 (nominal

Table 2
Relationship between covariates and log C-peptide

	log C-peptide	Duration of diabetes	Age	Insulin	Glucose
log C-peptide	1	−0.17**	−0.06	0.14**	0.04
Duration of diabetes		1	0.31**	0.08*	0.10*
Age			1	0.02	−0.08
Insulin				1	0.009
Glucose					1
Insulin treatment					
Yes	−0.12 ± 0.88	11.00 ± 9.83	52.41 ± 10.21	55.88 ± 46.31	223.10 ± 97.31
No	0.05 ± 0.6	6.49 ± 6.51	53.49 ± 10.91	15.85 ± 11.03	198.46 ± 89.54

* p-Value < 0.01.
** p-4alue < 0.001.

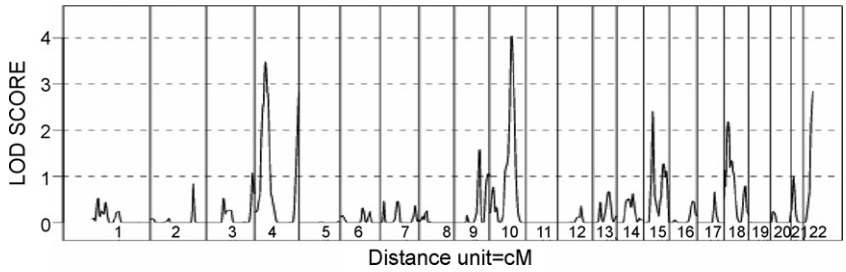

Fig. 1. Plot of genome-wide scan for log C-peptide from multipoint variance components linkage analysis with SOLAR on chromosome 1–22.

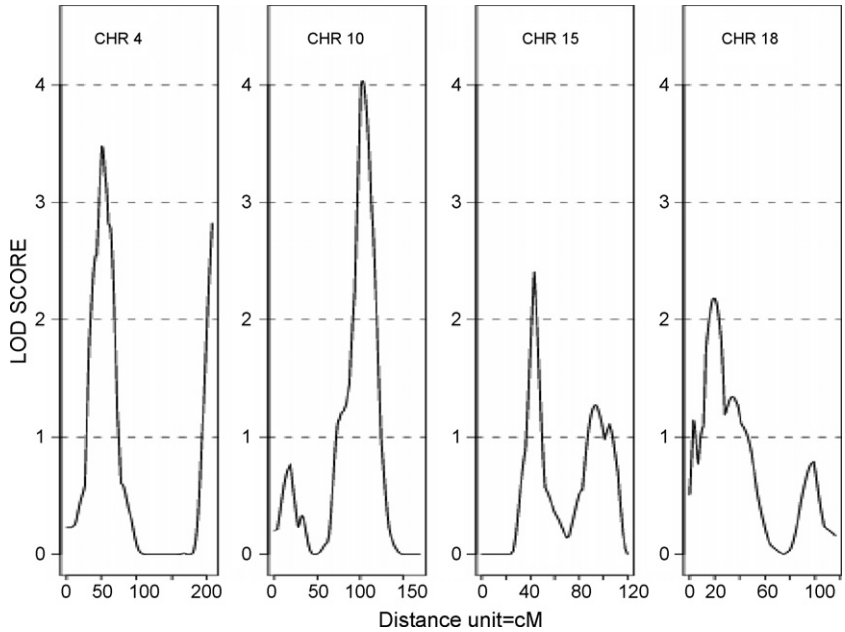

Fig. 2. Plot of log C-peptide from multipoint variance components linkage analysis on chromosome 4, 10, 15, and 18.

p-value = 0.000008 and empirical p-value = 0.0004). Another significant linkage region was located on 4p15 region near D4S2632 with a LOD score 3.48 (nominal p-value = 0.000031 and empirical p-value = 0.0013). One suggestive linkage region was identified on 15q14 near D15S659 with a LOD score 2.41 (nominal p-value = 0.000435 and empirical p-value = 0.0068) and another suggestive linkage region was observed on 18p11 near D18S976 with a LOD score 2.18 (nominal p-value = 0.000771 and empirical p-value = 0.0094). This study had 80% power to detect, with LOD score 3.0, a QTL which accounts for approximately 60% of the trait variance and with LOD score 2.0, approximately 52% of trait variance.

Table 3
Maximum LOD scores > 1.0 for log C-peptide in the AADM study

Chromosome	Nearest marker	Max location (cM)	Region (cM)	LOD score (nominal p-value)	Empirical p-value
3	D3S2418	216	215–218	1.08 (0.012910)	0.0532
4	D4S2632	51	40–65	3.48 (0.000031)	0.0013
9	D9S930	117	112–121	1.58 (0.003509)	0.0270
10	D10S2327	103	97–113	4.04 (0.000008)	0.0004
15	D15S659	43	38–48	2.41 (0.000435)	0.0068
15	D15S652	94	88–100	1.28 (0.007622)	0.0403
18	D18S976	20	11–28	2.18 (0.000771)	0.0094

4. Discussion

Physiologically, C-peptide has been shown to increase forearm muscle blood flow [17], to enhance oxygen uptake and capillary diffusion capacity in the exercising forearm [2]. Under diabetic condition C-peptide has been shown to: (1) redistribute micro-vascular skin blood flow in C-peptide negative patients [18]; (2) improve micro-vascular complications, such as diabetic nephropathy and diabetic neuropathy [1,3]; (3) attenuate Na^+–K^+-ATPase activity in different cell types [19,20]; and (4) augments the vasoconstrictor effects of neuropeptide Y in insulin-dependent patients. In all, there is growing evidence suggesting that pro-insulin C-peptide improves renal and nerve function as well as microcirculation in patients with insulin-dependent diabetes possibly by stimulating Na–K^+-ATPase activity [4,17,21].

Several genes on chromosome 18 have been suggested as metabolic disease candidates and one such gene, adenylate cyclase activating polypeptide 1 (ADCYAP1) previously known as PACAP, MIM 102980), is within our linkage region in 18p11 (multipoint empirical LOD score of 2.78) near marker C18S1781. The main function of the ADCYAP1 gene is the stimulation of insulin secretion in a glucose-dependent manner [21]. This action has been shown to be executed mainly through augmentation of the formation of cAMP and the stimulation of the uptake of calcium [21].

The peroxisome proliferative activated receptor, gamma, coactivator 1 (PPARGC1) gene is located in our linkage region on chromosome 4 (4p15, empirical LOD score = 2.53 peak at marker D4S2639). It has been well documented that many of the genes dysregulated in both diabetes and 'prediabetes' are regulated by PPARGC1 and nuclear respiratory factor (NRF)-dependent transcription [22]. Although the mechanism of action is not completely known, accumulating evidences suggest that PPARGC1 and NFR may play a central role in the pathogenesis of T2D [23,24].

Our strongest evidence of linkage was in the 10q23 region near D10S2327 with a LOD score of 4.04. Interestingly, this region also demonstrated significant linkage in a study of Mexican Americans with T2D [25]. This region on 10q harbors multiple candidate genes including PTEN, PPP1R5 and IDE for T2D [26,27]. Reduction of IDE activity by a pharmacological inhibitor increase islet amyloid polypeptide (amylin) accumulation and amylin-mediated cytotoxicity in cultured β-cells [27]. Two studies have demonstrated positive association between IDE and T2D [28,29]. In all, we plan to conduct fine mapping of our linkage regions especially the 10q23 region. In addition, we plan to further characterize important candidate genes in our linkage regions to evaluate their potential physiologic role in T2D.

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References

[1] B.L. Johansson, K. Borg, E. Fernqvisit-Forbes, T. Odergren, S. Remahl, J. Wahren, C-peptide improves autonomic nerve function in IDDM patients, Diabetologia 39 (1996) 687–695.

[2] B.L. Johansson, B. Linde, J. Wahren, Effects of C-peptide on blood flow, capillary diffusion capacity and glucose utilization in the exercising forearm of type 1 (insulin-dependent) diabetic patients, Diabetologia 35 (1992) 1151–1158.

[3] B.L. Johansson, S. Sjoberg, J. Wahren, The influence of human C-peptide on renal function and glucose utilization in type 1 (insulin-dependent) diabetic patients, Diabetologia 35 (1992) 121–128.

[4] T. Forest, T. Hunt, A. Pfutzner, J. Beyer, J. Wahren, New aspects on biological activity of C-peptide in IDDM patients, Exp. Clin. Endocrinol. Diabetes 106 (1998) 270–276.

[5] D. Dufayet, D. Raccah, M.F. Jannot, T. Coste, C. Rougerie, P. Vague, Erythrocyte Na/K ATPase activity and diabetes: relationship with C-peptide level, Diabetologia 41 (1998) 1080–1084.

[6] T. Forest, D. Dufayet De La Tour, T. Kunt, A. Pfutzner, K. Goitom, T. Pohlmann, et al., Effect of proinsulin C-peptide on nitric oxide, microvascular blood flow and erythrocyte Na^+, K^+-ATPase activity in diabetes mellitus type 1, Clin. Sci. 98 (2000) 281–290.

[7] D. Raccah, C. Fabreguetts, J.P. Azulay, P. Vague, Erythrocyte Na^+–K^+-ATPase activity, metabolic control and neuropathy in IDDM patients, Diabetes Care 19 (1996) 564–568.

[8] D. Raccah, M.F. Lamotte-Jannot, T. Issautier, P. Wague, Effect of experimental diabetes on Na/K-ATPase activity in red blood cells, peripheral nerve and kidney, Diabetes Metab. 20 (1994) 271–274.

[9] C. Rotimi, G. Chen, A. Adeyemo, P. Furbert-Harris, D. Guass, J. Zhou, et al., A genome-wide scan for type 2 diabetes susceptibility genes in West Africans, Diabetes 53 (2004) 838–841.

[10] N.C. Rotimi, G. Dunston, K. Berg, O. Akinsete, A. Amoah, S. Owusu, et al., In search of susceptibility genes for type 2 diabetes in West Africa. The design and results of the first phase of the AADM Study, AEP 11 (2001) 51–58.

[11] S.A.G.E., Statistical Analysis for Genetic Epidemiology, Release 4.0, Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve University, Cleveland, OH, 2000

[12] J.R. O'Chonnell, D.E. Weeks, A program for identifying genotype incompatibilities in linkage analysis, Am. J. Hum. 63 (1998) 259–266.
[13] Weir B., Genetic Data Analysis II: methods for discrete population genetic data, Sinauer Associates, Sunderland, MA, 1996.
[14] J.R. Fernandez, C. Etzel, T.M. Beasley, S. Shete, C.I. Amos, D.B. Allison, Improving the power of sib pair quantitative trait loci detection by phenotype winsorization, Hum. Hered. 53 (2002) 59–67.
[15] B.D. Allison, C.M. Neale, R. Zannolli, N. Schork, I.C. Amos, J. Blangero, Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trail loci-mapping procedure, Am. J. Hum. 65 (1999) 531–544.
[16] E. Lander, L. Kruglyak, Genetic dissection of complex trait: guidelines for interpreting and reporting linkage results, Nat. Genet. 11 (1995) 241–247.
[17] B.L. Johansson, J. Pernow, C-peptide potentiates the vasoconstrictor effect of neuropeptide Y in insulin-dependent diabetic patients, Acta Physiol. Scand. 165 (1) (1999) 39–44.
[18] T. Forst, T. Kunt, T. Pohlmann, K. Goitom, M. Engelbach, T. Beyer, et al., Biological activity of C-peptide on the skin microcirculation in patients with insulin-dependent diabetes mellitus, J. Clin. Invest. 101 (1998) 2036–2041.
[19] H.P. Wald, P. Scherzer, R. Rasch, M.M. Popovtxer, Renal tubular Na–K-ATPase in diabetes mellitus: relationship to metabolic abnormality, Am. J. Physiol. (1993) E96–E101.
[20] P. Vague, D. Dufayet, T. Coste, C. Moriscot, M.F. Jannot, D. Raccah, Association of diabetic neuropathy with Na–K-ATPase gene polymorphism, Diabetologia 40 (1997) 506–511.
[21] K. Filipsson, M. Kvist-Reimer, B. Ahren, The neuropeptide pituitary adenylate cyclase-activating polypeptide and islet function, Diabetes 50 (2001) 1959–1969.
[22] M.E. Patti, A.J. Butte, S. Crunkhorn, K. Cusi, R. Berria, S. Kashyap, et al., Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1, PNAS 100 (2003) 8466–8471.
[23] Y.L. Muller, C. Bogardus, O. Pedersen, L. Baier, A Gly482Ser missense mutation in the peroxisome proliferator-activated receptor r coactivator-1 is associated with altered lipid oxidation and early insulin secretion in Pima Indians, Diabetes 52 (2003) 895–898.
[24] M.E. Patti, Gene expression in humans with diabetes and prediabetes: what have we learned about diabetes pathophysiology? Curr. Opin. Clin. Metab. Care 7 (4) (2004) 383–390.
[25] R. Duggirala, J. Blangero, L. Almasy, T.D. Dyer, K.L. Williams, R.J. Leach, et al., Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans, Am. J. Hum. Genet. 64 (1999) 1127–1140.
[26] L. Hansen, J.N. Jensen, C.T. Ekstrom, H. Vestergaard, T. Hansen, O. Pedersen, Studies of variability in the PETN gene among Danish Caucasian patients with type 2 diabetes mellitus, Diabetologia 44 (2001) 237–240.
[27] R. Sladek, G. Rocheleau, J. Rung, C. Dina, L. Shen, D. Serre, et al., A genome-wide association study identifies novel risk loci for type 2 diabetes, Nature 445 (2007) 881–885.
[28] C.J. Groves, S. Wiltshire, D. Smedley, K.R. Owen, T.M. Frayling, M. Walker, et al., Association and haplotype analysis of the insulin-degrading enzyme (IDE) gene, a strong positional and biological candidate for type 2 diabetes susceptibility, Diabetes 52 (2003) 1300–1305.
[29] S. Karamohamed, S. Demissie, J. Volcjak, C. Liu, N. Heard-Costa, J. Liu, et al., Polymorphisms in the insulin-degrading enzyme gene are associated with type 2 diabetes in men from the NHLBI Framingham heart study, Diabetes 52 (2003) 1562–1567.