Variation in \textit{CHI3LI} in Relation to Type 2 Diabetes and Related Quantitative Traits

Camilla Noelle Rathcke\textsuperscript{1,4}, Johan Holmkvist\textsuperscript{2}, Torben Jørgensen\textsuperscript{3}, Knut Borch-Johnsen\textsuperscript{4}, Torben Hansen\textsuperscript{2}, Oluf Borbye Pedersen\textsuperscript{2,5,6}, Henrik Vestergaard\textsuperscript{1,6}

\textsuperscript{1} Department of Endocrinology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark, \textsuperscript{2} Hagedorn Research Institute, Gentofte, Denmark, \textsuperscript{3} Research Centre for Prevention and Health, Glostrup Hospital, University of Copenhagen, Copenhagen, Denmark, \textsuperscript{4} Steno Diabetes Center, Gentofte, Denmark, \textsuperscript{5} Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark, \textsuperscript{6} Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Abstract

\textbf{Background:} \textit{CHI3LI} encoding the inflammatory glycoprotein YKL-40 is located on chromosome 1q32.1. YKL-40 is involved in inflammatory processes and patients with Type 2 Diabetes (T2D) have elevated circulating YKL-40 levels which correlate with their level of insulin resistance. Interestingly, it has been reported that rs10399931 (−329 G/A) of \textit{CHI3LI} contributes to the inter-individual plasma YKL-40 levels in patients with sarcoidosis, and that rs4950928 (−131 C/G) is a susceptibility polymorphism for asthma and a decline in lung function. We hypothesized that single nucleotide polymorphisms (SNPs) or haplotypes thereof the \textit{CHI3LI} locus might influence risk of T2D. The aim of the present study was to investigate the putative association between SNPs and haplotype blocks of \textit{CHI3LI} and T2D and T2D related quantitative traits.

\textbf{Methods/Principal Findings:} Eleven SNPs of \textit{CHI3LI} were genotyped in 6514 individuals from the Inter99 cohort and 2924 individuals from the outpatient clinic at Steno Diabetes Center. In case-control studies a total of 2345 T2D patients and 5302 individuals with a normal glucose tolerance test were examined. We found no association between rs10399931 (OR, 0.98 (CI, 0.88–1.10), p = 0.76), rs4950928 (0.98 (0.87–1.10), p = 0.68) or any of the other SNPs with T2D. Similarly, we found no significant association between any of the 11 tgSNPs and T2D related quantitative traits, all p > 0.14. None of the identified haplotype blocks of \textit{CHI3LI} showed any association with T2D, all p > 0.16.

\textbf{Conclusions/Significance:} None of the examined SNPs or haplotype blocks of \textit{CHI3LI} showed any association with T2D or T2D related quantitative traits. Estimates of insulin resistance and dysregulated glucose homeostasis in T2D do not seem to be accounted for by the examined variations of \textit{CHI3LI}.

Introduction

The primary molecular defects in Type 2 diabetes (T2D) remain largely unknown, but it is widely recognized that both genetic and environmental risk factors have interacting roles in the pathogenesis and the progression of the disease. However, recent discoveries have led to a progress in the understanding of the molecular genetic background of T2D brought forward by genome-wide association (GWA) studies which have dramatically increased the genetic background of T2D brought forward by genome-wide association (GWA) studies which have dramatically increased the number of validated type 2 diabetes loci with modest impact on relative diabetes risk [1–3]. Several loci of interest may be located in the region of human chromosome 1q21–24 which shows replicated linkage to T2D in at least eight populations including North European Caucasian families, Pima Indians and Old Order Amish [1]. Furthermore, linkage analysis has also provided evidence for linkage of the metabolic syndrome (central obesity, dyslipidemia, hyperglycemia and hypertension) to 1q23–31 [4]. These regions as well as other closely related regions of chromosome 1 are under intense search for identification of T2D susceptibility genes.

Chitinase 3-like 1 (\textit{CHI3LI}) encoding the inflammatory glycoprotein YKL-40 is located in a phylogenetically highly conserved area on chromosome 1q32.1. YKL-40 is involved in inflammatory processes and extracellular matrix remodelling including angiogenesis and functions as a growth factor for several cell types [5]. Studies have shown, that patients with T2D have elevated plasma YKL-40 levels [6,7] which correlate with insulin resistance and with their diabetic lipid profile, but not with estimates of glycemic regulation [6]. Also patients with type 1 diabetes (T1D) have elevated YKL-40 levels which increase with increasing levels of albuminuria [8].

The \textit{CHI3LI} promoter variant −329 G/A (rs10399931) associates with the inter-individual plasma YKL-40 levels in patients with sarcoidosis [9]. Furthermore, the functional \textit{CHI3LI} promoter SNP −131C→G (rs4950928) increases the mRNA transcript levels coding for the YKL-40 protein in a genome wide
study of gene expression in Epstein-Barr virus-transformed lymphoblastoid cells from children with asthma [10] and is shown to be predictive of asthma, bronchial hyperresponsiveness and reduced lung function [11]. Since CHI3LI is a susceptibility gene for asthma with elevated circulating YKL-40 levels as a biomarker, it is possible that one of these single nucleotide polymorphisms (SNPs) or another gene variation with which they could be in tight linkage disequilibrium with may be functional in relation to T2D.

Applying a HapMap-based tagSNP approach the objective of the present study was to investigate the putative association of common variation in the CHI3LI locus with T2D, obesity and several T2D associated quantitative traits.

Methods

Ethics Statement

Informed written consent was obtained from all participants before participation. The study was approved by the Ethical Committees of Copenhagen and Aarhus and was in accordance with the principle of the Helsinki Declaration II.

Study population

A total of 11 tagSNPs (including the rs10399931 and rs4950928) covering all haplotype blocks in CHI3LI locus was genotyped. Genotyping was performed in 9438 individuals including Inter99, a population-based sample of unrelated, middle-aged Danish individuals living in the greater Copenhagen area, and 2924 individuals recruited from the outpatient clinic at Steno Diabetes Center.

The Inter99 study population has been described in detail previously [12]. In brief, both participants from the Inter99 cohort and individuals recruited from the outpatient clinic were characterized by an oral glucose tolerance test (OGTT). Impaired glucose homeostasis (IGF, IGT and diabetes) was defined according to WHO criteria after a 75 g OGTT [13] giving a classification of normal glucose tolerance (NGT; n = 5302), impaired fasting glycaemia (IGF; n = 540), impaired glucose tolerance (IGT; n = 830) or screen-detected untreated T2D (SDU DM; n = 294). Missing data was seen in 421 individuals (4.5%) and a total of 2091 individuals had known T2D. The participants were investigated for an association between genotype and quantitative metabolic traits; individuals with known type 2 diabetes were excluded from these analyses. All participants were of Danish nationality.

Biochemical and anthropometric measurements

Height and body weight were measured in light indoor clothes and without shoes, and BMI was calculated as weight (kg) divided by height (m)². Waist circumference was measured in the standing position midway between the iliac crest and the lower costal margin and hip circumference at its maximum.

In both Inter99- and Steno Diabetes Center participants, blood samples were drawn in the fasting state after a 12-h overnight fast. Plasma glucose was analysed by a glucose oxidase method (Granutest, Merck, Darmstadt, Germany). HbA1C was measured by ion-exchange high performance liquid chromatography (normal reference range: 4.1–6.4%) and serum insulin (excluding des(31, 32) and intact proinsulin) was measured using the AutoDELFIA insulin kit (Perkin-Elmer/Wallac, Turku, Finland). Serum C-peptide concentrations were measured by a time-resolved fluorimunnoassay (AutoDELFIA C-peptide kit; Perkin-Elmer/Wallac, Turku, Finland).

Serum triglyceride and total and HDL-cholesterol were analysed using enzymatic colorimetric methods (GPO-PAP and CHOD-PAP, Roche Molecular Biochemicals, Mannheim, Germany). HOMA-IR was calculated as fasting plasma glucose [mmol/l] multiplied by fasting serum insulin [pmol/l] and divided by 22.5. Insulinogenic index at 30 min was calculated as fasting serum insulin subtracted from plasma insulin at 30 min [pmol/l] divided by plasma glucose at 30 min [mmol/l].

Genotyping

SNPs in a region 22 kb upstream and 10 kb downstream of CHI3LI were chosen from the HapMap project (www.hapmap.org). Haplotype tagSNPs were selected from the HapMap data using TAGGER [14]. TAGGER was used with a 5% MAF cut off, agressive tagging (r² > 0.8). Two haplotype blocks with 2 and 5 SNPs, respectively, and with similar structures in both patients with T2D and in controls were identified (Figure 1). The other tagSNPs were not included in the haplotype block analyses since they were not part of any block. Genotyping was performed using TaqMan allelic discrimination (Kbioscience, Herts, UK) with a success rate >97.0%. Discordance was 0% as judged from re-genotyping of 953 random duplicate samples. Genotype distribution obeyed Hardy Weinberg Equilibrium (HWE), all p>0.25.

Statistical analyses

Logistic regression analyses were used to analyse differences in allele and genotype frequencies between case and control subjects; analyses were adjusted for age, sex and BMI where appropriate. The statistical analyses were performed using RGui version 2.5.0 (available at http://www.r-project.org). The haplotype block analyses were analysed for an association with T2D after adjustment for age, sex and BMI. Haplotype analyses were performed using PLINK [15]. P-values are not adjusted for multiple testing.

Results

Clinical characteristics for the Inter99 cohort and the individuals recruited at Steno Diabetes Center are presented in Table 1. A total of 2345 T2D patients and 5302 individuals with a normal glucose tolerance test were identified.

Overall we found no association of rs10399931, rs4950928 or any of the other tagSNPs of CHI3LI with T2D after adjustment for age, sex, BMI and glucose tolerance, all p>0.25 (Table 2). In similar analyses, none of the tagSNPs were associated with impaired fasting glucose (IGF) or impaired glucose tolerance (IGT), although a borderline protective association with IGT was detected for rs12123883 (Table S1 and S2).

Table 3 and 4 show the multiadjusted association of rs10399931 and rs4950928 with BMI and other T2D related quantitative traits. Although a borderline protective association with IGT was found for rs10399931, no other tagSNPs showed any associations (data not shown).

The two identified haplotype blocks of CHI3LI did not show any association with T2D (Table 5).

Discussion

This is a large-scale and to our knowledge the first study of variation in CHI3LI in relation to T2D and pre-diabetic quantitative traits. CHI3LI encodes the inflammatory protein YKL-40 which previously has been found to be elevated in plasma...
from patients with T2D [6,7] and from patients with T1D where it also correlates with increasing levels of albuminuria [8]. In our study, we have examined 11 tagSNPs covering the common variation (MAF>5%) in CHI3L1 including the SNPs rs10399931 and rs4950928 which previously have been found to contribute to interindividual variations in circulating YKL-40 levels in patients with sarcoidosis [9] and asthma [11], respectively. Furthermore, CHI3L1 has been found to be a susceptibility gene for asthma, since rs4950928 is predictive of asthma, bronchial hyperresponsiveness and reduced lung function.

A total of 9438 individuals have been examined, but no significant association of rs10399931, rs4950928 or any of the other tagSNPs of CHI3L1 were found with T2D or any of the intermediate T2D related quantitative traits. A borderline protective association with IGT was detected for rs12123883, but rs12123883 is a rare SNP with only few homozygotes and the protective association is most likely a chance finding. Furthermore, the p-value would not withstand correction for multiple testing considering the number of tests performed. On the same line, none of the two identified haplotype blocks showed any association with T2D.

In association analyses of rs10399931 and SNPs analysed in the DGI genome wide association scan study, rs10399931 is in LD with rs4950929 ($r^2 = 0.74$) and rs946263 ($r^2 = 0.78$). Other correlations had $r^2$-values<0.05. In the DGI genome wide association scan study, no significant associations of rs4950929 with any of the type 2 diabetic phenotypic traits were found, but the rs946263 G-allele was nominally found to be associated with T2D ($p = 0.027$) [16]. rs946263, which is a synonymous SNP located −9639 base pairs from the translational start site in

---

**Table 1. Clinical data for study sample.**

|                    | Complete sample | SDC sample | Inter99 cohort |
|--------------------|-----------------|------------|----------------|
| N (m/w)            | 9438 (4834/4604)| 2924 (1665/1259) | 6514 (3169/3345) |
| NGT                | 5302 (2456/2846) | 734 (334/400)  | 4568 (2122/2446) |
| IFG                | 540 (391/149)   | 32 (18/14)    | 508 (373/135)    |
| IGT                | 830 (414/416)   | 123 (66/57)   | 707 (348/359)    |
| SDU DM             | 294 (187/107)   | 39 (27/12)    | 255 (160/95)     |
| Known DM           | 2051 (1259/801) | 1946 (1200/755) | 105 (59/46)      |
| NGT age (years)    | 47±9            | 58±9         | 45±8            |
| NGT BMI (kg/m²)    | 25.6±9.1        | 25.9±8.7     | 25.5±4.1        |
| T2D age (years)    | 60±12           | 62±11        | 51±7            |
| T2D BMI (kg/m²)    | 30.1±5.5        | 30.0±5.5     | 30.4±5.6        |
| T2D HbA1c (%)      | 7.8±1.7         | 8.1±1.6      | 7.1±1.7         |

Data is mean±S.D. SDC, Steno Diabetes Center; N (m/w), number (men/women); NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; SDU DM, screen detected untreated diabetes mellitus; T2D, Type 2 diabetes; BMI, Body Mass Index; HbA1c, hemoglobin A1c. doi:10.1371/journal.pone.0005469.t001

---

Figure 1. Linkage disequilibrium (LD) pattern of the investigated variations of CHI3L1.
doi:10.1371/journal.pone.0005469.g001
**CHI3L1**, is also in perfect LD \((r^2 = 1.0)\) with rs4950928, which accounts for approximately 10% of the variance in plasma YKL-40 levels in asthma patients [11]. Beside the LD of rs10399931 and rs4950928 with rs946263, none of the examined SNPs or haplotype blocks of **CHI3L1** seem to be of any significance for the T2D diagnosis or any of the T2D related quantitative traits. Since the encoded inflammatory protein, YKL-40, of **CHI3L1** seems to be both a marker of inflammation and endothelial dysfunction [5,17] it is possible that it is the endothelial dysfunction and the later micro- and macrovascular complications of T2D that accounts for the elevated YKL-40 levels in T2D patients. In endothelial dysfunction, elevated YKL-40 levels seem to play a role in relation to cell migration, reorganization and tissue remodelling as a response to endothelial damage [18–20].

The possible association between YKL-40 and vascular damage is supported by the finding of an independent association between elevated circulating YKL-40 levels and increasing levels of albuminuria in T1D patients [8]. Elevated plasma YKL-40 is also found to be associated with the presence and extent of coronary artery disease as assessed by coronary angiography [21] and just recently, YKL-40 levels have been found to be elevated in patients with myocardial infarction [22]. Furthermore, increasing YKL-40 levels do predict cardiovascular mortality in individuals without known DM or CHD after adjustment for known CV risk factors and markers [7].

Substantial evidence supports the notion that endothelial dysfunction and low-grade inflammation play a major pathophysiological role in the development of insulin resistance and the

| SNP         | Allele | MAF   | Genotype | Genotype distribution | Additive model | \(p\) value |
|-------------|--------|-------|----------|-----------------------|----------------|-------------|
| rs883125    | C/G    | 15.6  | CC       | 3546 (71.8) 1574 (71.0) | 0.98 (0.86–1.12) | 0.78        |
|             |        |       | GG       | 1280 (25.9) 591 (26.7) |                |             |
|             |        |       | CC       | 114 (2.3) 51 (2.3) |                |             |
| rs880633    | C/T    | 46.3  | CC       | 1470 (30.0) 645 (29.4) | 1.00 (0.90–1.10) | 0.96        |
|             |        |       | CT       | 2416 (49.3) 1077 (48.8) |                |             |
|             |        |       | TT       | 1018 (20.7) 483 (21.8) |                |             |
| rs4950928   | C/G    | 20.4  | CC       | 3159 (63.6) 1402 (63.4) | 0.98 (0.87–1.10) | 0.68        |
|             |        |       | GG       | 1612 (32.4) 716 (32.4) |                |             |
|             |        |       | CC       | 197 (4.0) 93 (4.2) |                |             |
| rs10399931  | C/T    | 23.7  | CC       | 2894 (58.8) 1287 (58.4) | 0.98 (0.88–1.10) | 0.76        |
|             |        |       | CT       | 1754 (35.6) 791 (35.9) |                |             |
|             |        |       | TT       | 273 (5.6) 126 (5.7) |                |             |
| rs6691378   | G/A    | 12.6  | GG       | 3840 (77.7) 1690 (76.4) | 1.08 (0.94–1.25) | 0.29        |
|             |        |       | GA       | 1030 (20.9) 487 (22.0) |                |             |
|             |        |       | AA       | 69 (1.4) 34 (1.6) |                |             |
| rs4950930   | G/A    | 4.0   | GG       | 4512 (91.6) 2053 (92.5) | 0.96 (0.76–1.20) | 0.70        |
|             |        |       | GA       | 399 (8.1) 157 (7.1) |                |             |
|             |        |       | AA       | 13 (0.3) 10 (0.5) |                |             |
| rs12123883  | T/C    | 7.4   | TT       | 4235 (85.5) 1906 (85.7) | 0.95 (0.79–1.14) | 0.58        |
|             |        |       | TC       | 684 (13.8) 305 (13.7) |                |             |
|             |        |       | CC       | 37 (0.7) 13 (5.8) |                |             |
| rs2486064   | G/A    | 42.3  | GG       | 1636 (33.0) 742 (33.7) | 0.97 (0.87–1.07) | 0.48        |
|             |        |       | GA       | 2406 (48.6) 1057 (48.0) |                |             |
|             |        |       | AA       | 909 (18.4) 402 (18.3) |                |             |
| rs2886117   | G/A    | 12.9  | GG       | 3809 (76.6) 1679 (75.8) | 1.04 (0.90–1.20) | 0.59        |
|             |        |       | GA       | 1078 (21.7) 500 (22.6) |                |             |
|             |        |       | AA       | 84 (1.7) 36 (1.6) |                |             |
| rs872129    | A/G    | 7.2   | AA       | 4183 (84.5) 1910 (86.1) | 0.91 (0.76–1.09) | 0.32        |
|             |        |       | AG       | 730 (14.8) 298 (13.4) |                |             |
|             |        |       | GG       | 35 (0.7) 11 (0.5) |                |             |
| rs871799    | G/C    | 9.2   | GG       | 4028 (81.3) 1821 (82.3) | 0.96 (0.81–1.13) | 0.60        |
|             |        |       | GC       | 873 (17.6) 376 (17.0) |                |             |
|             |        |       | CC       | 54 (1.1) 16 (0.7) |                |             |

**Table 2.** Case control association studies of 2345 T2D patients and 5302 glucose tolerant control participants in relation to the 11 tgSNPs of **CHI3L1**.

**T2D**, type 2 diabetes; **SNP**, single nucleotide polymorphism; **MAF**, minor allele frequency; **NGT**, normal glucose tolerance; **OR (CI)**, odds ratio (confidence interval).

DOI:10.1371/journal.pone.0005469.t002
progression to manifest T2D [23–29]. We have previously shown, that YKL-40 levels are elevated in patients with T2D where it correlates with insulin resistance as assessed by the HOMA model and with levels of triglycerides and non-esterified fatty acids [6]. Since we found no association of any of the tgSNPs and surrogate markers of insulin resistance or the diabetic lipid profile in the present study, it does not seem to be any of these tgSNPs that participate in endothelial dysfunction and the development of T2D [23–25]. However, association studies between YKL-40 and ICAM-1, either at protein or transcript levels, have not been conducted. Neither have association studies of variations of CHI3L1 in relation to micro- or macrovascular conditions never been made.

In conclusion, both rs10399931 and rs49509028 are in high LD with rs946263 which is reported to associate with phenotypic traits of T2D in DGI [16]. However, neither rs1039991 nor rs4950928 nor any other tgSNPs of CHI3L1 which were investigated in the present study are associated with T2D or T2D related quantitative traits including estimates of insulin resistance and dysregulated glucose homeostasis in the examined approximately 9500 Danes. Further studies are required to determine YKL-40 levels as a marker of inflammation in the prediabetic state and the role of CHI3L1 in the pathogenesis of T2D.

### Supporting Information

**Table S1** Case control association studies of 540 individuals with impaired fasting glucose (IFG) and 5302 glucose tolerant control participants in relation to the 11 tgSNPs of CHI3L1. Found at: doi:10.1371/journal.pone.0005469.s001 (0.08 MB DOC)

**Table S2** Case control association studies of 830 individuals with impaired glucose tolerance (IGT) and 5302 glucose tolerant control participants in relation to the 11 tgSNPs of CHI3L1. Found at: doi:10.1371/journal.pone.0005469.s002 (0.08 MB DOC)
Table 5. CHI3L1 haplotype association studies in relation to T2D.

| SNPs            | Haplotype | Frequency (%) | total sample | p value |
|-----------------|-----------|---------------|--------------|---------|
| rs4950928 | GT        | 0.1999        |              | 0.71    |
| rs4950928 | CT        | 0.0345        |              | 0.98    |
| rs6691378 | CC        | 0.76398       |              | 0.74    |
| rs6691378 | GGTGA     | 0.00667       |              | 0.16    |
| rs6691378 | GATGG     | 0.04281       |              | 0.47    |
| rs6691378 | GCCAG     | 0.07954       |              | 0.54    |
| rs6691378 | GGTAG     | 0.35018       |              | 0.65    |
| rs6691378 | AGTGA     | 0.11792       |              | 0.46    |
| rs6691378 | GGTTAG    | 0.40448       |              | 0.38    |

Data show the association with T2D for the 2 haplotype blocks of 2 respectively 5 genotyped SNPs in CHI3L1. Haplotype tagSNPs were selected from the HapMap data using TAGGER.

T2D, type 2 diabetes; SNP, single nucleotide polymorphism.

doi:10.1371/journal.pone.0005469.0005

Acknowledgments

We wish to thank A. Forman, I.-L. Wantzin and M. Stendal for technical assistance and Michel Kristensen, Arne Nielsen and Grete Lademann for management and secretarial support.

References

1. McCarthy MI (2003) Growing evidence for diabetes susceptibility genes from genome scan data. Curr Diab Rep 3: 139–67.
2. McCarthy MI, Zeggini E (2006) Genetics of type 2 diabetes. Curr Diab Rep 6: 147–54.
3. McCarthy MI, Zeggini E (2007) Genome-wide association scans for Type 2 diabetes: new insights into biology and therapy. Trends Pharmacol Sci 28: 598–601.
4. Langefeld CD, Wagenknecht LE, Rotter JI, Williams AH, Hokanson JE, et al. (2006) Linkage of the metabolic syndrome to 1q23–q31 in Hispanic families: the Insulin Resistance Atherosclerosis Study Family Study. Diabetes 55: 1170–4.
5. Rathcke CN, Vestergaard H (2006) YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. Inflamm Res 55: 221–7.
6. Rathcke CN, Johansen JS, Vestergaard H (2006) YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance. Inflamm Res 55: 53–9.
7. Rathcke CN, Raymond I, Kistop C, Hildebrandt P, Faber J, et al. (2009) Low grade inflammation as measured by levels of YKL-40: Association with an increased overall and cardiovascular mortality rate in an elderly population. Int J Cardiol. In Press.
8. Rathcke CN, Persson F, Tarnow L, Rossing P, Vestergaard H (2009) YKL-40, a marker of inflammation and endothelial dysfunction, is elevated in patients with Type 1 Diabetes and increases with levels of albuminuria. Diabetes Care. In Press.
9. Kruit A, Gueters JC, Ruven HJ, van Mooevel CC, van den Bosch JM (2007) A CHI3L1 gene polymorphism is associated with serum levels of YKL-40, a novel sarcoidosis marker. Respir Med 101: 1563–71.
10. Dixo DL, Liang L, Mollfatt MF, Chen W, Heath S, et al. (2007) A genome-wide association study of global gene expression. Nat Genet 39: 1202–7.
11. Ober C, Tan Z, Sun Y, Possick JD, Pan L, et al. (2008) Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med 358: 1622–91.
12. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ihlen H, Glumer C, et al. (2003) A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. Eur J Clin Investig 33: 172–83.
13. Alpert KG, Zimmet PJ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part I: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15: 539–53.
14. de Bakker PI, Yelenosky R, Pe’er I, Gabriel SB, Daly MJ, et al. (2005) Efficiency and power in genetic association studies. Nat Genet 37: 1217–23.
15. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–75.
16. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, et al. (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316: 1331–6.
17. Johansen JS (2006) Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. Dan Med Bull 53: 172–209.
18. Shackleton LM, Mann DM, Mills AJ (1995) Identification of a 38-kDa heparin-binding glycoprotein (gp38k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodelling. J Biol Chem 270: 13076–83.
19. Malinou KM, Pounce L, Kleinman HK, Shackleton LM, Mills AJ (1999) Gp38k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. Exp Cell Res 250: 168–73.
20. Mills AJ, Hoyle M, Kent L (1996) In vitro expression of a 38,000 dalton heparin-binding glycoprotein by morphologically differentiated smooth muscle cells. J Cell Physiol 127: 666–72.
21. Kucur M, Isman FK, Karadas B, Vural VA, Tavsanoglu S (2007) Serum YKL-40 levels in patients with coronary artery disease. Coron Artery Dis 18: 391–6.
22. Noggaard C, Host NR, Christensen JF, Poulten SH, Egstrup K, et al. (2006) Serum levels of YKL-40 increases in patients with acute myocardial infarction. Coron Artery Dis 19: 257–63.
23. Meigs JB, Hu FB, Rifai N, Manson JE (2005) Markers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 291: 1978–86.
24. Pickup JC (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 27: 813–23.
25. Kolb H, Mandrup-Poulsen T (2005) An immune origin of type 2 diabetes? Diabetologia 48: 1038–50.
26. Eckel RH, Grundy SM, Zimmet PJ (2005) The metabolic syndrome. Lancet 365: 1415–28.
27. Festa A, Hanley AJ, Tracy RP, D’Agostino RB Sr, Haffner SM (2003) Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulin secretion. Circulation 108: 1022–30.
28. Rutter MR, Meigs JB, Sullivan LM, D’Agostino RB Sr, Wilson PW (2004) C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. Circulation 110: 380–5.
29. Ingelsson E, Hulthe J, Lind L (2006) Inflammatory markers in relation to insulin resistance and the metabolic syndrome. Eur J Clin Invest 36: 502–9.
30. Dupuis J, Larsson MG, Vasan RS, Massaro JM, Wilson PW, et al. (2005) Genome scan of systemic biomarkers of vascular inflammation in the Framingham Heart Study: evidence for susceptibility loci on 1q. Artherosclerosis 182: 307–14.
31. Puntasila I, Tofimov S, Kobylansky E, Livshis G (2002) Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. Cytokine 19: 138–46.

Author Contributions

Conceived and designed the experiments: CNR TJ KBJ TH OBP HV. Performed the experiments: CNR TJ KBJ TH OBP HV. Analyzed the data: CNR JH TH HV. Contributed reagents/materials/analysis tools: CNR [H TH HV. Wrote the paper: CNR. Critical revision of the manuscript: [H TJ KBJ TH OBP HV.
32. de Maat MP, Bladhjerg EM, Hjelmborg JB, Bathum L, Jespersen J, et al. (2004) Genetic influence on inflammation variables in the elderly. Arterioscler Thromb Vasc Biol 24: 2168–73.

33. Vickers MA, Green FR, Terry C, Mayosi BM, Julier C, et al. (2002) Genotype at a promoter polymorphism of the interleukin-6 gene is associated with baseline levels of plasma C-reactive protein. Cardiovasc Res 53: 1029–34.