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Interaction between Obesity and the NFKB1-94ins/delATTG Promoter Polymorphism in Relation to Incident Acute Coronary Syndrome: A Follow Up Study in Three Independent Cohorts

Jakob Gerhard Stegger1,2*, Erik Berg Schmidt1, Tina Landsvig Berentzen3, Anne Tjønneland4, Ulla Vogel5, Eric Rimm6,7, Thorkild I. A. Sørensen3,8, Kim Overvad1,9, Majken K. Jensen1,6

1 Department of Cardiology, Center for Cardiovascular Research, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark, 2 Department of Anesthesiology, Aalborg University Hospital, Aalborg, Denmark, 3 Institute of Preventive Medicine, Copenhagen University Hospital, Copenhagen, Denmark, 4 Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark, 5 National Research Centre for the Working Environment, Copenhagen, Denmark, 6 Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, Massachusetts, United States of America, 7 Department of Medicine, Channing Division of Network Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, 8 The Novo Nordisk Foundation Centre for Basic Metabolic Research, Section on Metabolic Genetics, Faculty of Medical and Health Sciences, University of Copenhagen, Copenhagen, Denmark, 9 Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark

Abstract

Introduction: The NF-κB transcription factor family regulates several genes encoding pro-inflammatory and anti-inflammatory proteins in adipose tissues and in atherosclerotic plaques. The deletion variant allele of the NFKB1-94ins/delATTG promoter polymorphism leads to lower transcript levels of the p50 subunit, and the variant allele has been associated with the risk of several inflammatory diseases as well as coronary heart disease where inflammation is important in the pathogenesis. The objective of this study was to explore the potential interaction between the NFKB1-94ins/delATTG promoter polymorphism and general, abdominal, and gluteofemoral obesity in relation to the risk of incident acute coronary syndrome (ACS) in three independent cohorts.

Methods and Results: The analyses were conducted in the Danish prospective study Diet, Cancer and Health and the two US based cohorts; Nurses’ Health Study and Health Professionals Follow-up Study. We conducted sex stratified analyses that included 1202 male and 708 female cases of incident ACS. We observed a positive association for general and abdominal obesity with risk of incident ACS, independent of genotype in both genders. Gluteofemoral obesity was negatively associated with ACS risk in women independent of genotype, whereas there was no clear association for men. We calculated the relative excess risk due to interaction (RERI) and observed a statistically significant excess risk among men jointly exposed to general or abdominal obesity and the variant allele of the NFKB1-94ATTG polymorphism, whereas there was a tendency towards sub-additivity for gluteofemoral obesity. The excess risks in all analyses were, however, small and could not clearly be demonstrated in women.

Conclusion: The variant allele of the NFKB1-94ins/delATTG promoter polymorphism did not substantially modify the association between obesity and incident ACS.


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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: Jakob.stegger@rm.dk

Introduction

Obesity is associated with a higher risk of acute coronary syndrome (ACS)[1–4]. However, many obese do not experience ACS, and other factors may affect the implications of obesity.

Adipose tissue is, in addition to being a storage organ for energy, an active endocrine organ that secretes both pro- and anti-inflammatory cytokines [5]. As obesity develops, the release of adipose tissue derived cytokines increases [6], and the resulting state of chronic low-grade inflammation may accelerate the...
Obesity and NF-κB Interaction in Relation to ACS

Baselined characteristics of the study participants are presented in Table 1. Compared to the controls both male and female cases in DCH appeared on average older, had a shorter education, fewer were never smokers, and drank less alcohol. In NHS and HPFS, age and smoking status were matching factors, and thus no differences were observed, but as in DCH, cases drank less alcohol than controls. In all studies, cases had a higher prevalence of hypertension, diabetes mellitus and hypercholesterolemia at baseline. Only few were current smokers in HPFS compared to NHS and DCH, and there were higher prevalence’s of hypertension, hypercholesterolemia and diabetes mellitus in the two US cohorts.

The primary anthropometric exposure variables are presented in Table 2. Among the controls the minor allele (the deletion variant) frequency was 37%–39%, and the NFKB1 genotype distribution was in Hardy-Weinberg equilibrium in all three studies (data not shown).

Both general obesity, measured as BMI, and abdominal obesity, measured as WC, were positively associated with ACS independent of genotype. Carrier status did not have a consistent effect on ACS risk among women with no consistent effect of genotype, whereas we observed no clear association in men.

We explored the possible interaction between obesity and carrier status by calculating the relative excess risk due to interaction (RERI) (Table S1). In men the combined effects of the NFKB1 - 94ins/delATTG promoter polymorphism and general or abdominal obesity showed a tendency towards positive interaction. Likewise, we found a tendency towards negative interaction, i.e. subadditivity, for the combined effect of the NFKB1 - 94ins/delATTG promoter polymorphism and gluteofemoral obesity. However, the estimates for the excess risk were small and could not clearly be demonstrated in women.

**Discussion**

In this large prospective study conducted in three independent cohorts, we observed no substantial interaction between obesity and the NFKB1 - 94ins/delATTG promoter polymorphism in relation to the risk of incident ACS; however, joint exposure to general or abdominal obesity and the variant allele was associated with the highest risk of ACS.

**Strengths and Limitations**

We achieved a high level of endpoint ascertainment as cases were validated by direct review of medical records. Likewise, data on anthropometric exposure variables were collected by trained study technicians in DCH, but in NHS and HPFS anthropometric measures were self reported, which could lead to measurement errors. However, all participants in NHS and HPFS were health workers, and the self reported measures have been validated, albeit there was a tendency towards underestimation of obesity measures [16]. Due to the subtle nature of atherosclerosis, reverse causation in relation to the anthropometric variables was a possibility, but we have previously in the total DCH cohort found similar associations for all anthropometric variables, when we conducted separate analyses in participants who experienced ACS within the first 2 years of follow up and in participants who experienced an ACS event after more than 2 years of follow up [3].

We adjusted for several potential confounders related to the associations between obesity and ACS, but did not include hypertension, diabetes mellitus and hypercholesterolemia in the analyses of the presented results as they can be seen as intermediate variables. Thus, their inclusion in multivariate analyses would restrict the outcome to associations through other pathways; however, analyses without these adjustments could lead to confounding from other causes to these intermediate variables. Crude analyses (Table S2), adjusted analyses (Table 3, 4 and 5) and analyses with additional adjustment for hypertension, diabetes mellitus and hypercholesterolemia (data not shown) provided similar results, and thus we do not believe that residual confounding explains our results.

**General Discussion**

The variant allele containing the deletion NFKB1-94ins/delATTG promoter polymorphism leads to lower levels of the p50 subunit, and this affects both the availability of the anti-inflammatory p50/p50 NF-κB homodimer and the pro-inflammatory p50/p65 NF-κB heterodimer. However, the combined effect of relatively low levels of both p50/p50 and p50/p65 will be pro-inflammatory, as low p50 levels intuitively will affect the concentration of p50/p65 more than the concentration of p50/p65. Furthermore, the p50/p65 homodimer blocks binding sites for the p65 subunit, and, thus, the level of the p50/p65 heterodimer will be relatively closer to normal, due to the
concurrent low levels of p50/p50 [11,12]. CRP transcription is controlled by the p50 homodimer. We have previously found lower levels of CRP protein in serum from carriers of the del-allele compared to ins-allele carriers, supporting the interpretation that the del-allele causes depletion of p50 homodimer [14]. This is also in line with the findings of increased levels of TNFalpha and IL-12 and reduced levels of IL-10 in NFKB1 knock-out mice [11]. However, the effect of the polymorphism may vary between different tissues. Abdominal adipose tissue secretes more anti-inflammatory cytokines than gluteofemoral adipose tissue [5]. Thus, in participants with high HC the net effect of the \(-94\)ins/delATTG promoter polymorphism may be a preferential depletion of anti-inflammatory cytokines, and a reduction in the protective effect of glucosefemoral fat deposits. Likewise, the combined effect of abdominal obesity and homozygote variant carrier status may be supra-additive due an excess secretion of pro-inflammatory cytokines as well as an increased inflammatory response to cytokines in atherosclerotic plaques. Even though our results does show a tendency of positive interaction for abdominal obesity and negative interaction for glucosefemoral obesity, and thus supports the above mentioned biological hypothesis, potential interaction between genetic variation in \(NFKB1\) and obesity was small and does not seem to be of clinical importance. However, due to the importance of inflammation the development of atherosclerosis further studies could be warranted to clarify the biological implications of genetic variations in genes involved in inflammation in relation to coronary heart disease.

In conclusion, we found no substantial interaction between the \(NFKB1\)-94ins/delATTG promoter polymorphism and general, abdominal, or glucosefemoral obesity.

Materials and Methods

Study Populations

The present study was based on three independent cohorts; the Danish prospective study Diet, Cancer and Health (DCH), and two US based cohorts; the Nurses’ Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). The Diet, Cancer and Health study was approved by the National Committee on Health Research Ethics [journal nr. (KF) 01-345/93] and the Danish Data Protection Agency. Written informed consent was obtained from all participants to search information from medical registers.

All studies have previously been described in detail[17–19], and thus only a brief description is presented here.

Diet, cancer and health. From November 1993 to May 1997, all men and women aged 50-64 years, born in Denmark, living in the greater Copenhagen or Aarhus areas, and with no previous cancer diagnosis registered in the Danish Cancer Registry were invited to participate in Diet, Cancer and Health (DCH). The study was approved by the National Committee on Health Research Ethics [journal nr. (KF) 01-345/93] and the Danish Data Protection Agency. Written informed consent was obtained from all participants to search information from medical registers.

To preserve biological material the present study was conducted as a case-cohort design based on a sex-stratified subcohort drawn...
were missing.

and participants for whom information on one or more variables excluded participants with an ACS diagnosis prior to baseline, randomly from the entire cohort. After case validation, we included participants with an ACS diagnosis prior to baseline, and participants for whom information on one or more variables were missing.

All anthropometric data were collected by trained technicians. Height was measured to the nearest 0.5 cm with the participants standing without shoes. Weight was measured using a digital scale with the participants wearing light clothing and recorded to the nearest 0.1 kg. Waist circumference (WC) was recorded to the standing without shoes. Height was measured to the nearest 0.5 cm with the participants wearing light clothing and recorded to the narrowest part between the lower rib and the iliac crest.

Blood samples were collected from each participant at baseline, and lymphocytes were isolated and frozen within 2 h. For genotyping, DNA was isolated from frozen lymphocytes from cases and participants in the subcohort and the \textit{NFKB1}-94 ins/del promoter polymorphism was determined as previously described[14,20–22]. Controls were included in each run, and repeated genotyping of a random 10% subset yielded 100% identical genotypes [14].

Information on confounders was obtained at baseline through questionnaires including socio-demographic factors, lifestyle, medication and prevalent disease.

In Diet, Cancer and Health, ACS was defined as unstable angina pectoris and myocardial infarction (ICD-8:410–410.99 and ICD-10: I20.0, I21.0–I21.9). Potential cases were identified by linkage to the Danish National Patient Registry [23] and the Danish Causes of Death Registry using the Danish Civil Registration System, in which every Danish citizen is identified by a unique 10 digit number. All incident cases between baseline and ultimo 2003 were validated by review of medical records in accordance with the guidelines of the American Heart Association and the European Society of Cardiology for use in epidemiology [24]. Furthermore, participants with a sudden cardiac death diagnosis (ICD 8:427.27 or ICD 10: I46.0–I46.9) were accepted as cases, if the cardiac arrest at validation was believed to have been caused by ACS.

A total of 80,996 men and 79,729 women were invited, and 27,148 men (34%) and 29,863 women (37%) consented to participate. The sex stratified cohort sample consisted of 1869 male and 224 female cases, and the subcohort comprised 1695 participants. Participants in the subcohort may throughout the paper be referred to as controls, even though the subcohort included 33 participants who had become cases.

The nurses’ health study and the health professionals follow-up study. The Nurses’ Health Study (NHS) was established in 1976 at the Channing Laboratory of the Brigham and Women’s Hospital, Boston, US. The study included married, female, registered nurses aged 30–55 years residing in one of 11 US states.

The Health Professionals Follow-up Study (HPFS) was established at the Harvard School of Public Health in 1986 with methods similar to that described for the NHS. It is a longitudinal
study among male health workers in the United States aged 40–75 years at enrollment.

In both studies, the participants have received follow-up questionnaires biennially to update information on lifestyle factors, body weight, and newly diagnosed illnesses. Self-reported information on height and weight was obtained at baseline, and self-reported measures of WC and HC only were obtained once in 1986/1987) and covariate data derived from questionnaires administered at blood draw (1990 in the NHS and 1994 in the HPFS), with missing information substituted from previous questionnaires [16,18]. Similarly, blood samples were collected from participants free of cardiovascular disease and cancer. Whole blood samples were centrifuged and stored in cryotubes as plasma,uffy coat, and red blood cells. DNA was extracted from the buffy coat fraction of centrifuged blood with the QLAmp Blood Kit (Qiagen, Chatsworth, CA, USA). The genotypes were determined by Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA, USA) as previously described [14,25].

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For the present study, we used nested case-control designs. Blood was donated from 32,826 Nurses and 18,224 HPFS participants. Between blood draw and June 2004, 512 female incident cases of ACS were identified in NHS, and 454 male cases were identified in HPFS. Using risk-set sampling, controls were selected randomly and matched in a 2:1 ratio on age, smoking, and month of blood return, among participants who were free of cardiovascular disease at the time ACS was diagnosed in the case patient [27].

After exclusions of participants for whom information was missing on the NFKB1 genotype, body mass index and covariate data, the NHS comprised 471 cases of incident ACS and 922 controls, and the HPFS comprised 427 cases and 875 controls. However, information on WC and HC was not available for all participants, and thus analyses regarding WC and HC only included 426 cases and 679 controls in NHS and 382 cases and 821 controls in HPFS.

### Table 3. RR* with 95% confidence interval in brackets for the combined effect of general obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome.

<table>
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<th>HPFS ²</th>
<th>Metaanalysis</th>
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<tr>
<td></td>
<td>NFKB1</td>
<td>NFKB1</td>
<td>NFKB1</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25 kg/m²</td>
<td>1.00 (ref.)</td>
<td>1.14 (0.85; 1.53)</td>
<td>1.10 (0.73; 1.66)</td>
</tr>
<tr>
<td>25–29 kg/m²</td>
<td>1.15 (0.86; 1.52)</td>
<td>1.40 (1.07; 1.84)</td>
<td>1.71 (1.24; 2.37)</td>
</tr>
<tr>
<td>&gt;29 kg/m²</td>
<td>1.49 (1.08;2.05)</td>
<td>1.61 (1.20;2.18)</td>
<td>1.98 (1.35;2.91)</td>
</tr>
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</table>

Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).

¹Adjusted for age, smoking status, alcohol consumption, physical activity, and educational level. Women also adjusted for menopausal status.

²Adjusted for age, smoking status, alcohol consumption, and physical activity. Women also adjusted for menopausal status and hormone replacement therapy.

*RR estimated by Cox proportional hazards regression in DCH and logistic regression in NHS and HPFS.

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25–29 and among cases with the DCH cohort as reference (Men: BMI
categorical variables with 3 levels according to the distribution
power, measures of obesity were included in the analyses as
the WHO classification, but to achieve the best possible statistical
anthropometric measures were described in tables according to
and gluteofemoral obesity, respectively. The distribution of
expressed as WC and HC mutually adjusted describing abdominal
measure of general obesity, and body fat distribution was
delayed entry of the participants, the incidence rate ratios in DCH
observation time was censored by death of other causes than ACS,
were analyzed by weighted Cox's proportional hazards regression
in accordance with the case-cohort design and to allow for
emigration or study end at December 31st 2003. Due to the nested
Statistical Analyses
In accordance with the case-cohort design and to allow for
delayed entry of the participants, the incidence rate ratios in DCH
were analyzed by weighted Cox’s proportional hazards regression
models with age as the underlying time variable [28,29]. The
observation time was censored by death of other causes than ACS,
emigration or study end at December 31st 2003. Due to the nested
case-control design, incidence rate ratios were estimated by logistic
regression models in NHS and HPFS. Crude conditional and
unconditional analyses (adjusted for matching factors) showed very
similar results (data not shown). However, in the conditional
analyses a significant number of cases where missing a match due
to missing exposure/covariate information, and thus we chose to
provide results from unconditional analyses.
We used body mass index (BMI, calculated as the weight in
kilograms divided by the square of the height in meters) as a
measure of general obesity, and body fat distribution was
expressed as WC and HC mutually adjusted describing abdominal
and gluteofemoral obesity, respectively. The distribution of
anthropometric measures were described in tables according to the
WHO classification, but to achieve the best possible statistical
power, measures of obesity were included in the analyses as
categorical variables with 3 levels according to the distribution
among cases with the DCH cohort as reference [Men: BMI <25,
25–29 and ≥29 kg/m²; WC <94, 94–102 and >102 cm; HC
<98, 98–103.5 and >103.5 cm. Women: BMI <24, 24–28 and
≥28 kg/m²; WC <80, 80–91 and >91 cm; HC <98, 98–104
and >104 cm).
In DCH, we performed separate analyses in men and women
and adjusted for potential confounding from age, smoking status,
alcohol intake, physical activity (expressed as hours per week of
strenuous activities) educational level, and for men menstrual
status. In NHS and HPFS we adjusted for age, smoking status,
alcohol intake, and physical activity (expressed in terms of
metabolic equivalent (MET)-hours). In NHS we furthermore
adjusted for menstrual status and hormone replacement therapy.
Hypertension, diabetes mellitus, and hypercholesterolemia are
possible intermediate variables in analyses of anthropometry and
ACS, and therefore, they were not included in the main analyses.
Deviation from an additive model was explored as the relative
excess risk due to interaction (RERI) and calculated as suggested
by Rothman [30] and Hosmer and Lemeshow [31].
To combine the estimates from the three study populations, we
used the weighted average of the log rate ratios with weights
according to the study-specific variance. A similar model on
original scale was used to calculate the combined estimates of the
relative excess risk due to interaction.
The proportionality assumptions of the Cox proportional
hazards models were evaluated graphically by log-minus-log plots.
All analyses were performed using Stata version 11.2 (StataCorp
LP, College Station, TX) and SAS version 9 (SAS Institute Inc.,
Cary, NC).

Table 4. RR* with 95% confidence interval in brackets for the combined effect of abdominal obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Women</th>
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<tr>
<td></td>
<td>WC</td>
<td>HC</td>
</tr>
<tr>
<td>&lt;80 cm</td>
<td></td>
<td></td>
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<tr>
<td>80–91 cm</td>
<td></td>
<td></td>
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<tr>
<td>&gt;91 cm</td>
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Adjusted for age, smoking status, alcohol consumption, physical activity, educational level, and hip circumference. Women also adjusted for menopausal status.

Adjusted for age, smoking status, alcohol consumption, physical activity, and hip circumference. Women also adjusted for menopausal status and hormone replacement therapy.

*RR estimated by Cox proportional hazards regression in DCH and logistic regression in NHS and HPFS.

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Supporting Information

Table S1

RR* with 95% confidence interval in brackets for the combined effect of obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome. Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).

Table S2

Crude analyses. RR* with 95% confidence interval in brackets for the combined effect of obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome. Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).

Acknowledgments

The authors would like to thank the Danish Cancer Society, the Danish Heart Foundation, the Danish Obesity Research Centre (DanORC), Gene-diet Interactions in Obesity (GENDINOB), and the Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome (HEPADIP). We would like to thank Hardeep Ranu and Pati Soule from the DF/HCC Genotyping Core for genotyping.

Author Contributions

Conceived and designed the experiments: JGS, EBS, KO, AT, UV, ER, MKJ, TIAS. Performed the experiments: JGS, KO, AT, UV. Analyzed the data: JGS, KO, TLB, UV, AT, ER, MKJ. Contributed reagents/materials/analysis tools: JGS, KO, AT, UV, ER, MKJ. Wrote the paper: JGS, EBS, TLB, AT, UV, ER, TIAS, KO, MKJ.

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