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Genetic variants in the DNA repair gene *NEIL3* and the risk of myocardial infarction in a nested case–control study. The HUNT Study

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ABSTRACT

Background: Enhanced generation of reactive oxygen species and increased oxidative-induced DNA damage have been identified as possible contributors to atherosclerosis. The base excision repair (BER) pathway is the principal mechanism by which mammalian cells repair oxidative DNA damage. BER deficiency can potentially accelerate atherogenesis.
Methods: We evaluated the association of Single Nucleotide Polymorphisms (SNPs) in genes encoding four different BER proteins (NEIL3, OGG1, APEX1 and XRCC1) with the incidence of myocardial infarction in a nested case–control study among participants of the second survey of the HUNT Study. The study population included 1624 cases and 4087 age- and sex-matched controls.
Results: For the *NEIL3* SNP rs12645561, the TT genotype was associated with increased risk of MI (OR 1.47, 95% CI 1.02–2.12, p uncorrected for multiple comparisons = 0.04) both in the genotypic test (compared to the CC genotype) and in the recessive genetic model (compared to the CC and CT genotypes combined). For the other two *NEIL3* SNPs (rs10013040 and rs1395479) and for the SNPs of *OGG1* (rs1052133), *APEX1* (rs1878703) and *XRCC1* (rs25489) we observed no association with risk of myocardial infarction.
Conclusion: We found that the *NEIL3* rs12645561 SNP TT genotype was associated with increased risk of myocardial infarction. If confirmed in other studies, this association may suggest a possible role of attenuated DNA repair, and NEIL3 in particular, in atherogenesis.

Abbreviations: BER, base excision repair; SNP, single nucleotide polymorphism; MI, myocardial infarction; ROS, reactive oxygen species; AP, apurinic/apyrimidinic; BMI, body mass index; HUNT, Helseundersøkelsen i Nord-Trøndelag/Nord-Trøndelag Health Study; HDL, high-density lipoprotein; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease; MI, myocardial infarction.

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1. Introduction

Myocardial infarction (MI) due to coronary artery atherosclerosis is a leading cause of morbidity and mortality worldwide [1]. The underlying pathology of atherosclerosis constitutes a multifactorial process which involves the interaction of environmental and predisposing genetic risk factors, with the bidirectional interaction between lipids and inflammation as a major pathogenic characteristic [2,3]. Enhanced generation of reactive oxygen species (ROS) is also an important feature of atherosclerosis, induced by etiological risk factors such as smoking and metabolic disturbances as well as their common final pathway, inflammation. Although ROS generation is a fundamental component of cellular metabolism and signal transduction, enhanced ROS generation may induce inflammation, cellular damage and apoptosis, as well as DNA instability. Indeed, DNA damage, including oxidative-induced DNA damage in circulating leukocytes as well as within the atherosclerotic lesion, has been identified as a possible contributor to atherogenesis [4–9].

If ROS-induced damage of DNA is not counteracted, it may promote cellular damage and apoptosis within the atherosclerotic lesion, leading to plaque instability. Attenuated DNA repair, resulting in persistent cellular and tissue injury, may also be an important contributor to the non-resolving plaque inflammation that characterizes plaque progression. Base excision repair (BER) is the primary DNA repair pathway that corrects base lesions caused by oxidation, hydrolysis, deamination and alkylation. BER is also involved in the repair of single strand breaks [10,11]. Briefly, the BER pathway is characterized by five specific and sequential enzymatic steps. The first one involves the recognition and removal of the altered base by one of the eleven DNA glycosylases (e.g. *E.coli* endonuclease VIII-Like 3 [NEIL3] and 8-Oxoguanine glycosylase [OGG1]), which results in generation of an apurinic/apyrimidinic (AP) site intermediate. Subsequently, the newly generated AP site is incised by an AP endonuclease (i.e. APEX1) or an AP lyase. In the next steps, the ends are trimmed and the gap is filled by a DNA polymerase. Finally, in the last step, the nick is sealed by a DNA ligase, restoring the DNA [12]. X-ray repair cross-complementing protein 1 (XRCC1) is an essential scaffold protein which is required for the coordination of the BER pathway through interaction of BER components, including APEX [13]. A deficiency in BER, giving rise to a decline in DNA repair capacity and increased DNA damage accumulation, may predispose to cancer development and aging [14–17]. However, a deficiency in the BER pathway could potentially also result in enhanced susceptibility to atherosclerosis and MI. Genetic variations affecting BER genes may therefore be logical candidates for examination in order to elucidate the role of BER in atherosclerotic disorders.

In the present study we evaluated the association of Single Nucleotide Polymorphisms (SNPs) in genes encoding four different BER proteins (NEIL3, OGG1, APEX1 and XRCC1) with the incidence of MI in a nested case–control study.

2. Methods

2.1. Study population

The HUNT Study (Nord-Trøndelag Health Study) constitutes a large database of clinical, anthropometric and socioeconomic information collected during three surveys of the population in Nord-Trøndelag County in Norway. Details of the study are available on the HUNT website (http://www.ntnu.no/hunt).

We conducted a nested case–control study among participants of the second survey of the HUNT Study (HUNT2). Between August 1995 and June 1997 all adults aged 20 years or older living in Nord-Trøndelag County were invited to participate in the HUNT2 survey. In total, 93,898 individuals were invited and 65,215 (69%) participated in the study. All the participants filled out a questionnaire and attended a clinical examination that also included blood sampling. Details about the study have been published elsewhere [18,19]. Among 65,215 participants, we excluded 5221 subjects that reported a history of MI, angina pectoris, or stroke at baseline and 1233 participants with missing values on the covariates, leaving 58,761 people eligible for inclusion.

2.1.1. Cases

After participating at the baseline examination, the cohort was followed-up for a first MI. The unique 11-digit identification number of every Norwegian citizen enabled linkage to information on incident MIs at the hospitals in Levanger and Namsos (Nord-Trøndelag Hospital Trust), which are the primary referral hospitals for this population. For the present study, the follow-up for MI was complete through 2008. MI cases were diagnosed according to the European Society of Cardiology/American College of Cardiology consensus guideline [20], and the diagnostic criteria included symptoms of coronary ischemia, elevated serum levels of troponins and cardiac enzymes, and specified ECG changes.

2.1.2. Controls

Controls were selected by incidence density sampling, widely recommended for nested case–control studies. It implies that the same subject may have been sampled more than once as a control, and that individuals selected as controls at one time point may have become cases later on [21]. Prior to selection of controls there was no available information on whether the individuals had sufficient DNA for genotyping. Therefore we oversampled controls, and minimum 2 controls with available DNA were finally selected per case, matched for age at risk and sex. Use of age at risk as the time dimension allows a very precise adjustment for age and is recommended for epidemiological studies where the outcome is strongly age-dependent as is the case with MI [22].

2.2. Clinical information at baseline

The clinical examination was conducted by trained nurses and included standardized assessment of blood pressure, weight, height, and waist and hip circumferences. Systolic and diastolic blood pressures were measured with a Dinamap 845XT (Criticon/GE Healthcare) based on oscillometry, and the average of the second and third measurement was used in the analysis. Height and weight were recorded with participants wearing light clothes without shoes, and body mass index (BMI) was computed as weight (in kilograms) divided by the squared value of height (in meters). Information on health, life-style factors and medication was collected by means of a self-administered questionnaire.

Non-fasting serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured in fresh serum samples at Levanger Hospital, using enzymatic colorimetric methods with reagents from Boehringer Mannheim on a Hitachi 911 Autoanalyzer, as previously described [19].

2.3. Genotyping

DNA was extracted from blood samples that were drawn at baseline and stored at HUNT Biobank, Levanger, Norway. We preferably searched for SNPs with an estimated minor allele frequency of 1% or higher that were located in regulatory or coding regions. However, the majority of SNPs that potentially could inactivate the protein either had a too low expected minor allele frequency or was not