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Original article

Leukotriene C_4 synthase and ischemic cardiovascular disease and obstructive pulmonary disease in 13,000 individuals ☆

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ABSTRACT

Ischemic cardiovascular disease and obstructive pulmonary disease involve inflammation. Leukotrienes may be important pro-inflammatory mediators. We tested the hypothesis that the (-1072)G>A and (-444)A>C promoter polymorphisms of leukotriene C_4 synthase confer risk of transient ischemic attack (TIA), ischemic stroke, ischemic heart disease (IHD), asthma, and chronic obstructive pulmonary disease (COPD). We genotyped individuals from the Danish general population, the Copenhagen City Heart Study, and Danish patients with IHD/coronary atherosclerosis, the Copenhagen Ischemic Heart Disease Study. We used prospective (n=10,386), cross-sectional (n=10,386), and case-control (n=2392+5012) designs. Allele frequency was 0.07 for (-1072)A and 0.29 for (-444)C. Cumulative incidence for TIA was higher for (-1072)AA versus GG genotype (log-rank: $p<0.001$), and lower for (-444)CC versus AA genotype (log-rank: $p=0.03$). Cumulative incidence for ischemic stroke was also lower for (-444)CC versus AA genotype (log-rank: $p=0.04$). Multifactorially adjusted hazard ratios for TIA were 5.2(95% CI:1.9–14) for (-1072)AA versus GG genotype, and 0.4(0.2–1.0) for (-444)CC versus AA genotype. Corresponding values were 1.9 (0.7–5.2) and 0.7 (0.5–1.0) for ischemic stroke, and 0.8 (0.4–1.6) and 1.0 (0.9–1.2) for IHD. In the case-control study, corresponding multifactorially adjusted odds ratios for IHD/coronary atherosclerosis were 0.5 (0.2–1.3) and 1.2 (1.0–1.5). These genotypes were not associated with risk of asthma or COPD. Leukotriene C_4 synthase promoter genotypes influence risk of TIA and ischemic stroke, but not risk of IHD/coronary atherosclerosis, asthma, or COPD.

1. Introduction

We previously found that leukotriene C_4 synthase (-1072)AA genotype predicted increased risk of ischemic cerebrovascular disease, whereas (-444)CC genotype predicted decreased risk [1]. Because cysteinyl leukotrienes and thus leukotriene C_4 synthase are involved in inflammation, which could be the mechanism behind the association with ischemic cerebrovascular disease, and because IHD and obstructive pulmonary disease also are inflammatory diseases, these genotypes could also influence risk of IHD and obstructive pulmonary disease.

In IHD, the inflammation mediated by leukotrienes has been given a central role in all phases of the atherosclerotic disease process, from lesion initiation, to lesion progression and plaque rupture [2]. Furthermore, cysteinyl leukotrienes are believed to cause constriction of atherosclerotic arteries and thus reduce coronary blood flow [3]. Antileukotriene medications are not directly used in early prevention of IHD; however, in asthmatics taking antileukotriene medication, a reduced risk of ischemic heart disease has been observed [4].

Leukotriene formation is initiated by activation of cytosolic phospholipase A_2, resulting in arachidonic acid release from the nuclear membrane. Further metabolism of arachidonic acid to leukotrienes is achieved by 5-lipoxygenase together with an activating protein to leukotriene A_4. This unstable intermediate metabolite is converted to either leukotriene B_4 by hydrolysis, or to cysteinyl leukotrienes by conjugation with glutathione by leukotriene C_4 synthase [5].

Leukotriene C_4 synthase is the rate limiting enzyme of the 5-lipoxygenase pathway. The level of transcription of this protein therefore has profound influence on the level of cysteinyl leukotrienes synthesized. In asthmatics, enhanced transcriptional capacity of blood eosinophils to generate leukotriene C_4 synthase may be caused by cytokine IL-4 [6], but may also be caused by genetic polymorphisms in the promoter region of the gene. Two known promoter polymorphisms at position (-1072)G>A and (-444)A>C of the *LTC_4 synthase*

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gene coding for leukotriene C_4 synthase influence the level of transcription of leukotriene C_4 synthase in stimulated conditions [7,8]. Beside our own findings on risk of ischemic cerebrovascular disease [1], the (-444)CC vs AA genotype has also been associated with increased coronary calcium and carotid intima media thickness [9], and with increased risk of aspirin intolerant asthma [10].

We tested the hypothesis that the (-1072)G>A and (-444)A>C promoter polymorphisms of *LTC$_4$ synthase* confer risk of IHD/coronary atherosclerosis, asthma, and COPD, as well as risk of the two components of ischemic cerebrovascular disease, TIA and ischemic stroke. For this purpose, we genotyped 10,386 individuals from the Danish general population (the Copenhagen City Heart Study), and 2392 Danish patients with IHD/coronary atherosclerosis matched with controls (the Copenhagen Ischemic Heart Disease Study).

2. Materials and methods

2.1. Study design

2.1.1. Prospective studies

Five prospective studies on risk of TIA, ischemic stroke, IHD, asthma, and COPD were conducted within The Copenhagen City Heart Study. For TIA and ischemic stroke, the follow-up period went from 1976 through 2000 with up to 24 years of follow-up; while for IHD, asthma and COPD, the follow-up period went from 1976 through July 2007 with up to 31 years of follow-up. Individuals diagnosed with the relevant outcome before study entry were excluded from analysis.

2.1.2. Cross-sectional studies

Two cross-sectional studies on self-reported asthma and spirometry defined COPD were conducted within the Copenhagen City Heart Study.

2.1.3. Case-control study

A case-control study on IHD/coronary atherosclerosis was conducted with cases from the Copenhagen Ischemic Heart Disease Study and matched controls without cardiovascular disease from the Copenhagen City Heart Study.

2.2. Participants

Herlev Hospital and Danish Ethics Committees KF 100.2039/91, KA 93125, and KA 99039 approved the study. Informed consent was obtained from participants. Only white participants of Danish descent were included in the present studies.

2.2.1. The Copenhagen City Heart Study

This is a prospective cardiopulmonary study of the Danish general population initiated in 1976–1978 [11–13]. A total of 19,329 women and men stratified into 5-year age groups from 20 to 80 years and above were drawn randomly from the national Danish Civil Registration System for Copenhagen. Of those invited, 14,223 (74%) attended. Participants supplemented with new participants in the younger age groups were re-examined in 1981–1983, 1991–1994, and 2001–2003, and followed until July 2007. Follow-up was 100% complete. At the 1991–1994 and/or 2001–2003 examinations, 10,386 gave blood for DNA analysis. At each examination, participants completed a questionnaire on risk factors for cardiopulmonary disease.

2.2.2. The Copenhagen Ischemic Heart Disease Study

Patients with IHD/coronary atherosclerosis were identified among patients from the Copenhagen area referred for coronary angiography because of angina pectoris from 1991 through 2004 [14,15].

2.3. Genotyping

We genotyped for the *LTC$_4$ synthase* (-1072) G>A (rs3776944) and (-444)A>C (rs730012) polymorphisms using TaqMan assays (Applied Biosystems, Stockholm, Sweden) [1], For the (-1072)G>A genotype we used one pair of TaqMan probes (5′-VIC-TCTCCCTCGCCCCGC-3′ and 5′-FAM-TCTCCCTTGCCCCGC-3′), and one pair of PCR primers (5′-GCCCAACTGGGAAGGCT-3′ and 5′- CTGCCTGGAGTTCTGGGT-3′). For the (-444)A>C genotype we used one pair of TaqMan probes (5′-VIC-CTTATCTGTTCCCTGTCCC-3′ and 5′-FAM- CTGTTCCCGGTCCC-3′), and one pair of PCR primers (5′- CCGCAGAGGAGGGTTTGG-3′ and 5′-CAGGCTCCCGGCTAACTC-3′). The six different genotypes were confirmed by sequencing (Ref Sequence DNA: NM_145867). Because we performed 2 rounds of re-runs, the call rate for genotyping was >99.9%.

2.4. Other covariates

Alcohol drinkers consumed four units of alcohol weekly or more (12 g alcohol per unit). Smoking status was defined as never, former, or active smokers. Hypertension was use of anti-hypertensive medication, a systolic blood pressure ≥140 mm Hg, and/or a diastolic blood pressure ≥90 mm Hg. Diabetes mellitus was self-reported disease, use of insulin or oral hypoglycemic agents, and/or nonfasting plasma glucose >11 mmol/L. Physical inactivity was leisure time activity less than four hours weekly and predominantly sedentary work. Body mass index was weight (kg) divided by height squared (m^2). Information on atrial fibrillation (World Health Organization International Classification of Diseases, 8th and 10th revisions: codes 427.93, 427.94 and code I48.9) was gathered from the national Danish Hospital Discharge Registry and the national Danish Causes of Death Registry. Furthermore, for the Copenhagen City Heart Study atrial fibrillation was also diagnosed from electrocardiographic recordings obtained at all four study examinations, and confirmed by two independent reviewers [16].

2.5. Pulmonary function tests

At the 1976–1978 and 1981–1983 examinations, determinations of forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were performed using an electronic spirometer (model N 403; Monaghan, Littleton, CO, USA), whereas at the 1991–1994 and 2001–2003 examinations, a dry wedge spirometer was used (Vitalograph, Maidenhead, England). Each spirometry procedure was performed in triplicate, and results were accepted only if the variation between 2 of these was less than 5%. The highest measurements of FEV_1 and FVC were used as absolute values (in milliliters) or as a percentage of predicted values.

2.6. Endpoints

2.6.1. TIA and ischemic stroke

Information on diagnoses of cerebrovascular disease (ICD-8 codes 431 to 438 and ICD-10 codes I61-I69+G45) was gathered from the national Danish Patient Registry and the national Danish Causes of Death Registry. For each person registered with cerebrovascular disease, hospital records were requested. To also include nonfatal nonhospitalized ischemic stroke patients, the participants were asked at the study examinations whether they previously had a stroke. If a person answered “yes”, further information was obtained from that person's general practitioner. Experienced neurologists reviewed all potential cases [17]. Possible stroke events (hospitalized as well as nonhospitalized) were validated using the World Health Organization definition of stroke: an acute disturbance of focal or global cerebral function with symptoms lasting longer than 24 hours or leading to death with presumably no other reasons than of