Life Sciences 85 (2009) 316–321

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Clinical factors such as B-type natriuretic peptide link to factor VII, endothelial NO synthase and estrogen receptor α polymorphism in elderly women

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ARTICLE INFO

Article history:
Received 9 March 2009
Accepted 18 June 2009

Keywords:
Natriuretic peptide
Estrogen receptor
Factor V Leiden
Endothelial nitric oxide synthase
Single nucleotide polymorphism
Thrombosis

ABSTRACT

Aims: This study evaluated the presence of genetic mutations in relation to thrombosis or atherosclerosis in elderly women.
Main methods: This is an observational study of 93 Japanese women with a mean age of 80.9 years recruited from outpatient clinics of Nagoya University and its related hospitals. Ten single nucleotide polymorphisms (SNPs) were studied. Each gene studied acts in or is related to either blood coagulation (factor V Leiden, prothrombin G20210A, factor XIII Val34Leu, factor VII Arg353Gln, MTHFR C677T, β-fibrinogen G-455A, PAI-1 4G/5G), metabolic syndrome-related pathways (PPARα Leu162Val), or endothelium/estrogen system (eNOS Glu298Asp, ERα IVS1-401). SNPs were analyzed for their relation to clinical values including lipids, B-type natriuretic peptide (BNP), fasting plasma glucose, tumor necrosis factor-α, interleukin-6, cyclic GMP, and nitric oxide metabolites.
Key findings: Comparisons between the distributions of different genotypes and clinical values showed three relationships. First, factor VII Arg353Gln and HDL-cholesterol (HDL-C) were linked to Arg/Arg carriers at higher levels ($P=.049$). The HDL-C to LDL-cholesterol ratio supported this link ($P=.027$). Second, eNOS Glu298Asp and triglycerides were linked to Glu/Glu carriers at higher levels ($P=.031$). Third, ERα IVS1-401 and BNP were related to CC genotype at lower levels ($P=.031$). Additionally, the last two relations showed that genotype does not influence the demarcation line of biomarkers, but the plasma/serum levels of biomarkers instead.
Significance: Correlations of factor VII Arg353Gln with HDL-C and eNOS Glu298Asp with triglycerides are new findings. Polymorphisms in the endothelium/estrogen system and the heart failure marker BNP are also correlated, with ERα IVS1-401 being the first identified marker. SNPs may be helpful for understanding the pathophysiology of atherosclerotic diseases in elderly women.

Introduction

A thrombogenic state is an important risk factor for atherosclerotic diseases such as myocardial infarction and stroke. Thrombosis has been suggested to be an adverse effect associated with postmenopausal hormone-replacement therapy (HRT) (Fisher et al. 1998; Ettinger et al. 1999; Rosendaal et al. 2002).

Genetic mutation factors have been associated with an elevated risk associated with thrombosis. For example, factor V Leiden (also known as Arg506Gln, R506Q, or G1691A) has been associated with a 6.69-fold enhancement of the risk posed by thrombosis (Cushman et al. 2004). This single nucleotide polymorphism (SNP) is a guanine (G) to adenine (A) transition at the second base of the codon for amino acid position 506 in exon 10 of the factor V (symbol; F5) gene. In total, 5% of Caucasians are carriers of this SNP, which is known to make the protein resistant to inactivation by activated protein C (Bertina et al. 1994). This SNP is found in 20% of venous thrombosis patients (Rosendaal et al. 1995). Regional differences exist; East Asians do not carry factor V Leiden (Rees et al. 1995), which could be one of the reasons for the lower incidence of thrombosis in East Asians, although this remains unclear. Another noted SNP seen in Caucasians is prothrombin G20210A, which is a G to A transition at position 20210 of the prothrombin (symbol; F2) gene. It is observed among 6% of

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0024-3205/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.lfs.2009.06.009

venous thrombosis patients (Poort et al. 1996). For these two blood clotting factors, a meta-analysis has found a moderate association with coronary disease (Ye et al. 2006), implying that, for these and other clotting factors, a genetic study should be performed.

Several SNPs related to thrombosis and atherosclerotic risk have been proposed. Factor XIII Val34Leu shows a protective effect against venous thromboembolism (Mikkola et al. 1994; Wells et al. 2006). Factor VII Arg353Gln (R353Q or G10976A) leads to a reduction of its protein levels with a lower risk of cardiovascular disease (Green et al. 1991; Lane et al. 1992; Hunault et al. 1997; Girelli et al. 2000). MTHFR C677T leads to higher homocysteine levels and is thus a risk factor for coronary artery diseases (Frosst et al. 1995; Ma et al. 1996). β-fibrinogen G-455A (HaeIII) is associated with higher plasma fibrinogen levels (Thomas et al. 1991; Iso et al. 1995; van' t Hooft et al. 1999). PAI-1 4G/5G, which is the 4G allele related to elevation of circulating PAI-1, is associated with cardiovascular disease (Dawson et al. 1991; Eriksson et al. 1995; Hoekstra et al. 2004). PPARα Leu162Val (L162V) is associated with increased transcriptional activation of itself and the elevation of serum lipid levels (Vohl et al. 2000; Sapone et al. 2000). eNOS Glu298Asp (E298D) is associated with the possibility of impaired endothelial function (Philip et al. 1999; Guzik et al. 2001). Lastly, ERα IVS1-401 (IVS1-397 or PvuII), which is a transition in the intervening sequence 1 at position-401, is associated with cardiovascular disease (Herrington et al. 2002b; Nordström et al. 2003).

In atherosclerotic lesions, lipid accumulation in the plaque intima (Kramsch et al. 1971), increased cytokine levels (Rus et al. 1991, 1996), and an increased BNP level are all detected (Casco et al. 2002). It is believed that nitrite and nitrate, which are metabolites of nitric oxide (NOx) in vascular disease, are important factors that should be assessed (Palmer et al. 1987). BNP and NO elevates intracellular cGMP level (Palmer et al. 1987; Chinkers et al. 1989), indicating the importance of this second messenger. From a clinical point of view, it is important to identify biomarkers that can be used to predict both disease and longevity (Nomura et al. 2002; Hayashi et al. 2007). However, the use of SNPs as biomarkers is not well understood to date. Our aim is to evaluate genetic factors in elderly women in order to identify biomarkers that correlate with SNPs. The experimental design of this report was restricted to an East Asian population that does not possess factor V Leiden, because it is an easier model for screening candidate factors associated with thrombosis or atherosclerotic risk. In addition, analyses focusing on postmenopausal elderly women are rarely performed, and thus may result in an improved fundamental understanding of older people.

Material and methods

Subjects

We enrolled 104 Japanese female subjects over 60 years of age who were admitted to the Department of Geriatrics in an outpatient clinic of Nagoya University Hospital, Nagoya City (Japan), between May 2004 and January 2005. All subjects gave written informed consent. Proper authorization for the study was obtained from the Ethics Committee of the Nagoya University Graduate School of Medicine. As the data of serum or plasma collection or amount of assay for DNA analyses were insufficient in 11 patients, we finally analyzed the data of 93 patients.

Serum or plasma collection and measurement

Serum or plasma was collected from fasting blood samples after centrifugation at 3000 rpm at 4 °C, and stocked at −30 °C until measurement. BNP and cGMP concentrations were derived from the blood sample analysis (SRL Laboratories, Japan) as measured by a specific immunoassay. Tumor necrosis factor-α and interleukin-6 were measured using the Quantikine HS Kit (R & D Systems, USA). NOx was determined by high-performance liquid chromatography (Hayashi et al. 2007). Other clinical biochemical factors, such as LDL-C, HDL-C, and triglyceride levels, were also assessed.

DNA isolation and genotyping

DNA was isolated from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Düsseldorf, Germany) and genotyped with the Mutector Dual Well Test Kit (TrimGen, Maryland, USA) according to the manufacturer's instructions. The Mutector kit was designed for mutation detection among known nucleotide substitutions using a 96-well strip plate, and can confirm all three genotypes (wild, mutant homozygous, and heterozygous types). Both positive and negative controls were provided by the manufacturer. In brief, a complimentary detection primer is designed and immobilized on the wells whose 3' end terminates just before the target base (i.e. order made by offering the specified sequence information). The polymerase chain reaction product from the preceding step is added to wells together with labeled nucleotides and extension primer for either mutant or wild strand. Primer for mutant strand makes extension when the target base is mutant type but not wild, and vice versa. As a result, labeled nucleotides are incorporated and a colorimetric reaction is observed and measured by 405 nm on a microplate reader.

Selection of SNPs

All ten analyzed SNPs have referential SNP cluster identification numbers (RefSNP ID) provided by the NCBI (National Center for Biotechnology Information, Maryland). They are: factor V Leiden (rs6025), prothrombin G20210A (rs1799963), factor XIII Val34Leu (rs3024472), factor VII Arg353Gln (rs6046), MTHFR C677T (rs1801133), β-fibrinogen G-455A (rs1800790), PAI-1 (-675) 4G/5G (rs1799889), PPARα Leu162-Val (rs1800206), eNOS Asp298Glu (rs1799983), and ERα IVS1-401 (rs2234693).

Statistical analyses

The association of genotype distribution with clinical factors, represented as mean ± SEM, was analyzed using Microsoft Excel enhanced software with either an unpaired Student's *t*-test or a Mann–Whitney *U*-test, depending on histogram distribution. A chi-square test was used to describe the effect of genotype on biomarker levels

Table 1
All variables are presented as mean ± SEM.

Characteristic		On registration [$n=93$]
Age, years	60–64 [$n=4$]	80.95 ± 0.90
	65–74 [$n=16$]	
	75–84 [$n=36$]	
	85–94 [$n=36$]	
	95–99 [$n=1$]	
Total cholesterol, mg/dL		206.13 ± 4.62
LDL-C, mg/dL		120.73 ± 4.28
HDL-C, mg/dL		57.89 ± 2.05
Triglycerides, mg/dL		113.37 ± 5.60
Creatinine, mg/dL		0.84 ± 0.03
BNP, pg/mL		77.67 ± 8.17
Glucose, mg/dL		99.29 ± 3.52
TNF-α, pg/mL		3.96 ± 0.31
IL-6, pg/mL		7.08 ± 2.04
cGMP, pmol/mL		7.28 ± 0.40
NOx, μmol/L		55.38 ± 4.00
Hemoglobin, g/dL		11.89 ± 0.20

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BNP, B-type natriuretic peptide; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; cGMP, cyclic guanosine 5'-monophosphate; NOx, nitric oxide metabolites.