Thrombosis Research 136 (2015) 865–869

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

Integrin beta-3 genetic variants and risk of venous thromboembolism in colorectal cancer patients

Daniela Bianconi [a,1], Alexandra Schuler [a,1], Clemens Pausz [a], Angelika Geroldinger [b], Alexandra Kaider [b], Heinz-Josef Lenz [c], Gabriela Kornek [a], Werner Scheithauer [a], Christoph C. Zielinski [a], Ingrid Pabinger [a], Cihan Ay [a], Gerald W. Prager [a,*]

[a] Department of Internal Medicine I, Comprehensive Cancer Center Vienna, Medical University of Vienna, Austria
[b] Center for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Austria
[c] Norris Cancer Center, University of Southern California, Los Angeles, CA, USA

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 12 August 2015
Accepted 16 August 2015
Available online 28 August 2015

Keywords:
Single nucleotide polymorphism
Integrin β3
Venous thromboembolism risk
Colorectal cancer
rs3809565
Anticoagulant prophylaxis

ABSTRACT

Background: Integrin β3 is involved in tumor and endothelial cell biology as well as in platelet aggregation. Herein, we evaluated the predictive potential of three germline single nucleotide polymorphisms (SNPs) in the integrin β3 gene (rs3809865, rs5918 and rs4642) to predict the risk of venous thromboembolism (VTE) in colorectal cancer (CRC) patients, which is one of the leading causes of death among cancer patients.
Methods: 112 patients diagnosed with CRC enrolled in the prospective Vienna Cancer and Thrombosis Study (CATS) were assessed with a median follow-up of 46 months. DNA was isolated from venous blood samples and SNPs were analyzed by the PCR-RFLP method.
Results: VTE occurred in 12% (n = 13) of all patients. The SNPs rs5918 and rs4642 were not associated with VTE risk. For rs3809565, 23% (n = 11) of patients had the A/A genotype, 4% (n = 2) had the A/T genotype, but none (0%) had the T/T genotype. In the univariate analysis, patients with the A/A genotype had a significantly higher risk to develop VTE compared to the other polymorphisms ($P = 0.0005$ after Fine and Gray). In the multivariable analysis, the predictive value remained significant.
Conclusions: This study identified the rs3809865 A/A genotype as an independent risk factor for VTE in CRC patients. Our findings would help identify high risk patients and would be essential for tailored anticoagulant prophylaxis.

1. Introduction

Venous thromboembolism (VTE) is one of the leading causes of death in cancer patients causing additional life-threatening complications and significantly higher health care costs [1–3]. Specific cancer related patient characteristics such as tumor site, stage at diagnosis, histologic subtype, tumor grade or anticancer treatment with fluoropyrimidines ± bevacizumab or oxaliplatin can promote the development of VTE [4–8]. In colorectal cancer (CRC) patients the estimated risk of developing VTE within 2 years was reported to be 8.2% [9]. Patients with metastatic disease have a 5- to 13-fold higher risk of developing VTE compared to those with localized disease [10,11]. The pathogenesis that leads to a hypercoagulable state in cancer patients is complex and is mediated by various interactions of tumor cells with platelets and endothelial cells, affecting the clotting system [12,13].

Integrin receptors are heterodimeric cell adhesion proteins which consists of an α and β subunit [14]. Integrin β3 is essentially expressed on endothelial cells, platelets, osteoclasts and hematopoietic cells and corresponds to the group of integrins that binds to proteins containing the arginine-glycine-aspartic acid (RGD)-motif [15]. Integrin β3 can form heterodimers with the subunits αV and αIIb. Integrin αVβ3 is expressed by activated endothelial cells and tumor cells [16]. It promotes proangiogenic endothelial cell behavior, such as cell migration, invasion and survival, and is a major mediator of tumor angiogenesis, tumor growth and platelet aggregation [16–20]. Integrin αIIbβ3 is exclusively expressed on platelets and mediates platelet aggregation to promote thrombus formation [20,21].

The paramount role of integrin β3 in thrombus formation prompted us to investigate if there was a correlation between VTE in patients with CRC and the β3 integrin SNPs rs3809865, rs5918 and rs4642. The selection of these SNPs is based on a previous work [22], in which it was

Abbreviations: SNP, single nucleotide polymorphism; VTE, venous thromboembolism; CRC, colorectal cancer; RFLP, restriction fragment length polymorphism.

* Corresponding author at: Comprehensive Cancer Center Vienna, Medical University of Vienna, Waehringer Guertel 18–20, A-1090 Vienna, Austria.
E-mail addresses: cihan.ay@meduniwien.ac.at (C. Ay), gerald.prager@meduniwien.ac.at (G.W. Prager).

[1] D.B. and A.S. contributed equally to this study.

http://dx.doi.org/10.1016/j.thromres.2015.08.010

investigated if a comprehensive panel of germline SNPs of integrin genes could predict stage-specific time to tumor recurrence in colon cancer. In this post-hoc analysis, we retrospectively analyzed 112 patients recruited in the framework of the prospective Vienna Cancer and Thrombosis Study (CATS) for association of genetic variants in the integrin β3 gene with risk of VTE. 43% (n = 48) of all the patients had a rs3809865 A/A genotype, 45% (n = 50) a A/T genotype and 12% (n = 14) a T/T genotype. VTE occurred in 12% (n = 13) of all patients, 23% (n = 11) of A/A rs3809865 patients, in 4% (n = 2) of A/T patients, but none (0%) patients had the T/T rs3809865 genotype. rs5918 and rs4642 SNPs were not associated with risk of VTE in this population. This study identified the germline polymorphism A/A of rs3809865 as an independent risk factor for VTE in CRC patients. A single laboratory test stratifying high and low risk groups for developing VTE, could lead to prevention strategies, thus, improving quality of life, safe costs and decreasing mortality rates in these patients.

2. Methods

2.1. Patients and study design

112 CRC patients who entered the prospective CATS between February 2004 and June 2011 were analyzed. The study was performed in accordance with the Declaration of Helsinki and was approved by the ethical committee of the institution. Detailed information about the CATS study has been reported previously [23,24]. In brief, patients diagnosed with CRC gave informed consent before venous blood samples were drawn. The overall aim of CATS was defined to identify parameters and biomarkers to predict occurrence of VTE in cancer patients. While the CATS maximum observation period for VTE events is 2 years, the occurrence of objectively confirmed VTE in this analysis was retrospectively extended until August 2013. In CATS, study patients were not routinely screened for VTE, but symptomatic or fatal VTE were classified as events. Diagnosis of VTE was in all cases confirmed by objective medical imaging methods, such as duplex sonography or computer tomographic scans. Each event was discussed and evaluated by an independent adjudication committee. Non symptomatic events, such as accidentally detected VTE in a restaging examination, were considered as an event when the adjudication committee considered them to be of clinical significance. Patients with continuous oral anticoagulation were excluded and no routine thromboprophylaxis for VTE was performed in our study. However, thromboprophylaxis with low-molecular-weight-heparin (LMWH) was allowed in hospitalized patients or after surgery according to clinical practice guidelines. In addition to CATS, this analysis considered additional patient data such as anti-VEGF treatment.

2.2. Blood sampling and SNP genotyping

Venous blood samples were drawn into plasma vacuum tubes (Vacuette; Greiner Bio One, Austria) containing one-tenth volume sodium citrate stock solution at 0.129 mM. To obtain platelet-poor plasma, the citrated blood was centrifuged (ROTANTA/TRC; Hettich, Germany) at 1500 g for 15 minutes, and to obtain platelet free plasma a second centrifugation step (Eppendorf) at 13 400 g for 2 minutes was performed. Plasma aliquots were stored at −80 °C until they were assayed for PCR testing of three SNPs in integrin β3 gene. Samples were coded before laboratory analysis. During analysis researchers and technicians were unaware of the patients' characteristics at all times. Genotyping was performed applying a combined PCR-restriction fragment length polymorphism approach (PCR-RFLP). In brief, a short sequence including the site in question was amplified using primers binding 66–99 bps upstream and downstream resulting in PCR products 149–158 bps in size. Subsequently, the amplification product was digested using restriction enzymes, which were specifically chosen to cut or not at the site of the polymorphism in question according to genotype. After digestion, the resulting DNA-fragments were separated on an agarose gel. Consequently, lanes with bands in all three size ranges were considered heterozygote samples and lanes with only one band in the 150 bp region or two bands in the 60–100 bp region were considered to be homozygote in respect to the SNP in question. (Primers and enzymes are listed in supplemental Table S1). PCR assays were performed with 1.25 U DreamTaq™, 8 mM (total) dNTPs, 1x DreamTaq™ Green Buffer (Fermentas), 0.5 μM fw-primer and 0.5 μM rv-primer. Annealing temperatures were optimized in advance. Restriction digestions with SNP-specific restriction enzymes were performed according to the recommendations of the manufacturer. DNA-fragments were separated on a pre-stained (GelRED™, GenON) 4% agarose gel at 120 V for 30 min. and visualized on a BioRADChemiDOC XRS system.

2.3. Statistical analysis

Continuous variables were described with median and interquartile range (IQR); nominal variables were described by absolute numbers and percentage. SP-Selectin was compared between genotypes using t-tests on the logarithmised variable. The median follow-up time was estimated using the reverse Kaplan-Meier method [25]. The effects of sex, age, stage, metastatic site, tumor location, BMI and of the polymorphisms rs3809865, rs5918 and rs4642 on the occurrence of VTE was investigated in univariate, bias corrected Fine-Gray models [26] where death was considered as competing event. Due to the small number of events, we only considered multivariable Fine-Gray models including rs389086 and either sex, age, stage, metastatic site, tumor location and BMI. Since Fine-Gray models with death as competing risk do not handle time-dependent variables adequately [27], the effect of the time-dependent variable treatment with bevacizumab and of rs3809865 adjusted for treatment with bevacizumab was estimated in a bias corrected Cox-model where death was considered as censoring event. Here, we assumed that the effect of bevacizumab persisted 4 weeks after the end of therapy. We plotted crude cumulative incidence curves for each level of the polymorphism rs3809865, where death was considered as competing event. Chi-square goodness-of-fit tests were used to test the polymorphisms for Hardy-Weinberg Equilibrium and to compare for each polymorphism the minor allele frequency of our study population with the Global MAF based on 1000 Genomes. All analyses were carried out using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Study population

Baseline demographic and clinical characteristics of the study population are displayed in Table 1. One-hundred-twelve patients after diagnosis with CRC were prospectively observed. Median age at time of enrolment into CATS was 64 years (IQR, 57–71 years). Median observation time was 1399 days (IQR, 704–2197 days). 42 female patients (37%) and 70 male patients (63%) had been enrolled. Left sided primary tumors, including those originating from the splenic flexure, sigmoid, rectosigmoid junction, or rectum were recorded in 81 cases (72%), while right sided colon cancer was listed in 23 cases (21%). 3 patients (2%) were enrolled with two colorectal primary tumors and 5 (4%) patients were considered as CRC with non-recorded primary origin. Most patients (79%) had locally advanced (stage III) or metastatic disease (stage IV). During the observation period, 49 patients (44%) received the anti-VEGF antibody bevacizumab. Two patients were enrolled into blinded randomized controlled studies considering bevacizumab as the experimental treatment arm. Whether the two patients received bevacizumab is unknown; none of these patients, however, were diagnosed with VTE.