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Original Article

Genetic variation in the first-pass metabolism of ethinylestradiol, sex hormone binding globulin levels and venous thrombosis risk

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ABSTRACT

Background: Use of ethinylestradiol, one of the active ingredients in combined oral contraceptives, affects the incidence of venous thrombosis. To explain why some women develop thrombosis when using oral contraceptives and others do not, we hypothesized a role for the first-pass metabolism of ethinylestradiol in the liver. We set out to determine the association between genetic variation in the first-pass metabolism of ethinylestradiol, venous thrombosis risk and the effect on Sex-hormone-binding-globulin (SHBG) levels.
Methods: Premenopausal women were included from two case-control studies: LETS (103 cases; 159 controls) and MEGA (397 cases; 796 controls). Haplotype-tagging SNPs were selected in 11 candidate genes; COMT, CYP1A2, CYP2C9, CYP3A4, CYP3A5, SULT1A1, SULT1E1, UGT1A1, UGT1A3, UGT1A9, UGT2B7. Venous thrombosis risk was expressed as odds ratios (OR) with 95% confidence intervals (CI). For SHBG levels, mean differences with 95%CI were estimated in combined oral contraceptive-using control subjects from the MEGA study.
Results: Two copies of haplotype D in the UGT2B7 gene increased venous thrombosis risk (OR_{LETS}: 3.78; OR_{MEGA}: 2.61) as well as SHBG levels (mean difference 27.6 nmol/L, 95%CI: −61.7 to 116.9 compared with no copies) in oral contraceptive users and not in non-users. In oral contraceptive users, haplotype A and B in the CYP3A4 gene were associated with venous thrombosis risk, but not in non-users; however, the effect on SHBG levels was not directional with the risk. None of the other haplotypes were associated with venous thrombosis.
Conclusion: Genetic variation in the UGT2B7 gene may, in part, explain venous thrombosis risk in combined oral contraceptive users.

1. Introduction

Combined oral contraceptive use, containing an estrogen (i.e., ethinylestradiol) and a progestagen, increase venous thrombosis risk [1–4]. Over time the dose of ethinylestradiol was stepwise reduced from ≥100 μg to 50 μg and 30/20 μg, which resulted in the intended lowering in the risk of venous thrombosis [1,5,6]. The risk of venous thrombosis is the highest in the first three months of combined oral contraceptive use, i.e., about twelve-fold increased compared with non-users [7–9]. With extended use the risk remains approximately five-fold increased [9]. While some high-risk groups have been identified, i.e., women with prothrombotic genetic defects and women who are obese, it is largely unknown why oral contraceptive use leads to thrombosis in some women, and not in others.

Because they are taken orally, combined oral contraceptives are metabolized in the liver through the so-called first-pass metabolism. In the liver many coagulation factors are produced; therefore, we hypothesized that the first-pass metabolism of oral contraceptives, in particular of ethinylestradiol (Fig. 1), influences the risk of venous thrombosis, and that genetic variation in involved genes explains the difference in susceptibility between women. In general, the first-pass metabolism of drugs mainly involves conjugation and hydroxylation. Sulfonation and glucuronidation are both conjugation steps leading to inactive and water-soluble compounds which are excreted by the kidneys or the intestinal tract (via bile). The genes *SULT1A1* and *SULT1E1* code for sulfotransferases that are involved in sulfonation of ethinylestradiol [10–15] and the genes *UGT1A1*, *UGT1A3* and *UGT1A9* code for UDP-glucuronosyltransferases involved in the glucuronidation of ethinylestradiol [15–18]. Hydroxylation and subsequent methylation of the hydroxyl group lead to hydroxy-ethinylestradiol and methoxy-ethinylestradiol, respectively. The genes *CYP1A2*, *CYP2C9*, *CYP3A4* and *CYP3A5* code for enzymes involved in the hydroxylation step

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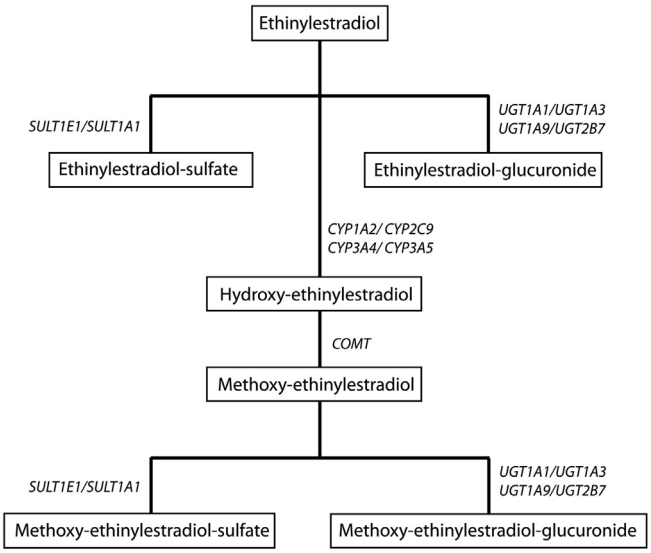

Fig. 1. The first-pass metablism of ethinylestradiol in the liver. Genes involved in this metabolism are depicted in italics.

[15,19–21] and the *COMT* gene codes for catechol O-methyltransferase involved in the methylation step [22]. The aforementioned conjugation steps are able to inactivate hydroxyl-ethinylestradiol and methoxy-ethinylestradiol.

Sex hormone binding globulin (SHBG) is a marker for the hormonal effects of combined oral contraceptives on venous thrombosis risk. SHBG is a hepatic plasma glycoprotein that binds the sex steroid hormones testosterone and 17β-estradiol, but not ethinylestradiol. Estrogens such as ethinylestradiol increase the synthesis of SHBG [23], while progestagens induce a decrease in SHBG levels depending on the type and dose [24,25]. The effect of a combined oral contraceptive on SHBG levels may be seen as the result of the stimulating effect of ethinylestradiol and the inhibiting effect of progestagen in the contraceptive [25].

The aim of this study was to explain differences in susceptibility to the prothrombotic effect of oral contraceptives by assessing genetic variation in the first-pass metabolism of ethinylestradiol in premenopausal women. Because the investigated enzymes are also involved in other metabolic pathways, results from non-users were evaluated to assess the specificity of the association with risk. Any risk associations with venous thrombosis observed in non-users, in whom the first-pass metabolism of ethinylestradiol is not activated, will be a reflection of genetic variation in the other pathways. Furthermore, genetic variation in the first-pass metabolism of ethinylestradiol was linked to an intermediate variable, SHBG levels, in combined oral contraceptive users. A priori, three criteria were established to determine whether a haplotype was associated with venous thrombosis through changes in the first-pass metabolism of ethinylestradiol, i.e., a similar association with venous thrombosis in combined oral contraceptive users in two independent studies (LETS and MEGA study), no association in non-users, and a direction of the effect on SHBG levels in accordance with the association with venous thrombosis risk.

2. Subjects and methods

2.1. Participants

Participants were selected from two case-control studies on venous thrombosis, i.e., the LETS (Leiden Thrombophilia Study) and the MEGA (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study. In the LETS study, participants with a first, symptomatic, objectively confirmed episode of deep venous thrombosis in the leg, younger than 70 years and without a known malignant disorder were enrolled between 1 January 1988 and 31 December 1992. As controls, acquaintances and partners of the patients were invited to participate. Details of the study have been described elsewhere [26]. In the MEGA study, participants with a first symptomatic deep venous thrombosis in the leg or arm or pulmonary embolism were recruited between 1 March 1999 and 31 August 2004. Controls were either the partners of the patients or recruited through random digit dialling (RDD). Details of the study have been described elsewhere [27]. Participants of both studies were asked to fill in a questionnaire and to provide a blood or buccal swab sample. The LETS and the MEGA study differed slightly in their in- and exclusion criteria. To increase homogeneity in the present analysis, only patients with a deep venous thrombosis of the leg were studied.

The population of interest consisted of premenopausal women younger than 50 years ($N_{LETS} = 347$; $N_{MEGA} = 2657$). Women who had any type of cancer ($N_{MEGA} = 63$), were hospitalized ($N_{LETS} = 22$; $N_{MEGA} = 357$), had undergone surgery ($N_{LETS} = 29$; $N_{MEGA} = 277$), suffered bone fractures ($N_{LETS} = 2$; $N_{MEGA} = 81$) or had soft-tissue injuries ($N_{LETS} = 0$; $N_{MEGA} = 529$) in the twelve months before the index date were excluded. We also excluded women who were pregnant ($N_{LETS} = 11$; $N_{MEGA} = 65$), were within four weeks postpartum ($N_{LETS} = 4$; $N_{MEGA} = 17$) or were using hormone replacement therapy ($N_{MEGA} = 14$) at the index date or had experienced a miscarriage ($N_{LETS} = 1$; $N_{MEGA} = 10$) in the twelve months before the index date. Oral contraceptive users were defined as users of a combined oral contraceptive at the index date (i.e., a contraceptive containing ethinylestradiol and a progestagen). Hence, in both studies women using a contraceptive without ethinylestradiol, e.g., progestagen-only pills, were excluded ($N_{LETS} = 2$; $N_{MEGA} = 26$). Women without a blood or buccal samples were also excluded ($N_{MEGA} = 366$). 500 patients and 955 controls were included in the current analysis (LETS: 103 cases and 159 controls; MEGA: 397 cases and 796 controls). In both studies, the sum of all exclusions does not add up to the total number of excluded participants because women could be exposed to one or more risk factors.

2.2. DNA preparation and SNP typing

Collection and processing of blood samples and buccal swabs and subsequent DNA isolation have been described previously [26,28]. To determine the haplotypes in the selected genes, the Genome Variation Server (GVS) was used. GVS incorporates information from HapMap and other sources and is sponsored by SeattleSNPs. Only SNPs with a minor allele frequency of 5% or more in white subjects were considered.

A total of 11 genes involved in ethinylestradiol metabolism were selected prior to genotyping, i.e., *COMT*, *CYP1A2*, *CYP2C9*, *CYP3A4*, *CYP3A5*, *SULT1A1*, *SULT1E1*, *UGT1A1*, *UGT1A3*, *UGT1A9*, and *UGT2B7* in which a total of 74 SNPs were selected (see Supplementary Table 1). Care was taken to select SNPs from each haplotype that enabled a clear distinction between highly related genes. The SNPs were either determined with the MassARRAY platform (Sequenom, San Diego, California, USA) according to manufacturer's protocols (Sequenom) or determined with the 5′ nuclease/Taqman assay (Assay-by-Design, Applied Biosystems, Foster City, California, USA). SNP rs28946889 was determined via restriction digest of the PCR product (details available on request). Genotyping determination was done blinded to the case/control status and study number. Five percent of the samples were repeated for allele-calling consistency, no discrepancies were found.

2.3. Laboratory measurement

SHBG levels (nmol/L) were measured with an immunometric assay (Immulite 2000 XPi; Siemens Healthcare Diagnostics, Tarrytown, NY, USA) in combined oral contraceptive users of the control group of the MEGA (plasma of the LETS subjects was no longer available). The sensitivity of the assay is 0.2 nmol/L and the log-term variation is 6% at levels of 5 nmol/L and 80 nmol/L. The within-assay variation is 3 to 4% and the

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