Thrombosis

Association of estrogen receptor-α gene polymorphisms with venous thrombosis

_Xba_I and _Pvu_II polymorphisms of the estrogen receptor-α gene (ERα) have been associated with several multifactorial diseases. We studied the distribution of ERα polymorphisms in patients with deep vein thrombosis. _Pvu_II PP and _Xba_I XX genotypes may be associated with an approximately 2-fold increased DVT risk of deep vein thrombosis in men.

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The effects of estrogens are mediated by estrogen receptors (ER) -α and –β, which are members of the nuclear hormone-receptor superfamily. Intronic polymorphisms in the regulatory region of the gene coding for ER-α have been associated with decreased bone mineral density,[1,2] increased risk of cardiovascular disease,[3] different responses of HDL cholesterol to estrogen replacement therapy[4] and increased susceptibility to atherosclerosis in men.[5] Based on the possible effects of ER-α polymorphisms on estrogen metabolism, on the clinical associations that have been described in the above mentioned studies,[1-5] on the association of estrogen therapy with increased risk of venous thromboembolism (VTE)[6] and on the relationship between atherosclerosis and VTE,[7] it is reasonable to suppose that ER-α polymorphisms might be independently associated with a multifactorial disease such as VTE, or may act synergistically with known risk factors. To investigate the possible association of ER-α with deep vein thrombosis (DVT), we studied the distribution of ER-α polymorphisms in 303 patients with a previous, objectively documented episode of proximal DVT and in 476 healthy controls, mostly recruited among friends of the index patients. All individuals underwent thrombophilia screening by established methods and their DNA was used to search for ER-α polymorphisms by restriction endonucleases *Pvu*II and *Xba*I as described elsewhere.[1] The presence of the restriction site was indicated with small letters (*p* or *x* respectively, for *Pvu*II and *Xba*I endonucleases), whereas capital letters (*P* or *X*) are used to denote its absence.

Table 1 shows the characteristics of the subjects enrolled in the study. The *Pvu*II PP genotype was significantly more prevalent in DVT patients than in controls (22% vs 15% p=0.02), while the observed different prevalence of *Xba*I XX (17% vs 12%) did not reach statistical significance. The crude odds ratio (OR) for DVT associated with genotypes *Pvu*II PP or *Xba*I XX was 1.6 (95% CI=1.1-2.3) or 1.5 (95% CI=0.9-2.2), respectively (Table 2); the association of genotype *Pvu*II PP with DVT risk remained statistically significant after adjustment for age, sex, factor V Leiden and prothrombin G20210A (OR=1.5, 95% CI=1.0-2.3) (Table 2). The *Pvu*II PP and *Xba*I XX genotypes were associated with DVT risk in men (adjusted OR=1.8, 95% CI=1.0-3.3, and OR=1.6, 95% CI=0.9-3.0 respectively), but not in women (Table 2), independently of whether or not they were taking oral contraceptives (*not shown*). A subanalysis of 142 men showed that the PP genotype was associated with a similar risk of DVT for idiopathic thrombosis (n=97; adjusted OR=1.9, 95% CI=0.9-3.7) and secondary events (n=45; adjusted OR=1.6, 95% CI=0.7-3.8). The *Pvu*II PP and *Xba*I XX genotypes were associated with prothrombin G20210A (but not with factor V Leiden) more frequently among cases than controls (29% vs 0%, p= 0.08; 23 % vs 0%, p=0.17), but this difference did not reach statistical significance due to the small number of subjects (n=46) with the prothrombin G20210A mutation.

To our knowledge, the association of ER-α *Pvu*II PP genotype with an approximately two-fold increased risk

Table 1. Demographic and clinical characteristics of the subjects enrolled in the study

	Cases	*Controls*
Women/men	158/145	272/204
Age, median (range), in years	42 (10-75)	47 (13-77)
Factor V Leiden#	54 (18%)	15 (3%)
Prothrombin G20210A#	35 (12%)	11 (2%)
Number of deep vein thromboses		
One	224 (74 %)	N.A.
More than one	79 (26 %)	N.A.
Circumstantial risk factors at 1st event		
None	155 (51 %)	N.A.
At least one	148 (49 %)	N.A.
Women/men	108/40	
Type of risk factor		
Oral contraceptive use	67 (62 %)§	66 (24%)^
Pregnancy or puerperium	19 (18 %)§	N.A.
Trauma or prolonged immobilization	36 (24 %)*	N.A.
Surgery	24 (16 %)*	N.A.
Antiphospholipid antibodies	2 (1%)*	Not tested

*N.A.: not applicable; #No patient with factor V Leiden or prothrombin G20210A had either the double defect or an association with any other thrombophilic defect; * percentage calculated on those with at least one risk factor (n=148); §number and percentage calculated only on women in reproductive age, excluding each from the other because they are mutually exclusive; ^percentage calculated on control women (n=272).*

Table 2. Distribution of genotypes *Pvu*II PP and *Xba*I XX in cases and controls and odds ratios for deep vein thrombosis.

	Cases	*Controls*	*Odds ratio (95% CI)*	*Odds ratio§ (95% CI)*
Pvu*II				
All subjects	66/303 (22%)	71/476 (15%)	1.6 (1.1-2.3)	1.5 (1.0-2.3)
Men	37/145 (26%)	31/204 (15%)	1.9 (1.1-3.2)	1.8 (1.0-3.3)
Women	29/158 (18%)	40/272 (15%)	1.3 (0.7-2.3)	1.3 (0.7-2.4)
Xba*I				
All subjects	50/303 (17%)	57/476 (12%)	1.5 (0.9-2.2)	1.4 (0.9-2.1)
Men	31/145 (21%)	27/204 (13%)	1.8 (1.0-3.1)	1.6 (0.9-3.0)
Women	19/158 (12%)	30/272 (11%)	1.1 (0.6-2.0)	1.1 (0.6-2.2)

**Genotypes dichotomized for the presence (Pp+pp or Xx+xx) [reference group] vs the absence (PP or XX) of the restriction site. §Adjusted for age, factor V Leiden, prothrombin G20210A and sex (when applicable).*

of DVT in men was never observed in previous studies. Our data do not allow us to clarify whether or not the PP genotype is a marker or a mediator of DVT. Based on the observations that estrogen therapies are associated with an increased risk of DVT[6,9] and that the *pp Pvu*II genotype is relatively hormone-insensitive,[1-2,4,8] one could consider it biologically plausible that the PP genotype, which is more sensitive than the pp genotype to sexual hormones, is associated with a heightened risk for DVT. The functional phenotype associated with the PP genotype may have clinical consequences only under conditions characterized by very low circulating levels of estrogens, such as in men, while it may be clinically irrelevant under conditions characterized by high circulating levels of estrogens, such as in fertile women. Due to the small number of subjects, it was not possible to study the association of the *Pvu*II PP genotype with DVT risk in post-menopausal women, in whom circulating estrogen levels are also low. The combined analysis of our data and those of a recent study that found an association of the PP genotype with a heightened risk for atherosclerosis and myocardial infarction in men[10] suggests that atherosclerosis and venous thromboembolism might have a common background, as was recently demonstrated in a large observational study.[7]

These results need to be confirmed in other studies that might also provide useful insights into the biochemical and physiopathological aspects of this association.

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