

## The factor V HR2 haplotype and the risk of venous thrombosis: a meta-analysis

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**Background and Objectives.** A complex haplotype of factor V gene (FV HR2) has been recently reported. FVHR2 possesses decreased co-factor activity to APC in the degradation of FVIIIa, and an increased ratio of the more procoagulant isoform FV1 compared to FV2. Contrasting results on whether the haplotype induces a significant risk of venous thromboembolism (VTE) have been reported.

**Design and Methods.** It has been surmised that FVHR2 enhances the risk of VTE carried by FV Leiden. We carried out a meta-analysis of the reported studies on the role of HR2 haplotype in inducing a risk of VTE and the influence of the polymorphism on the risk carried by patients with FV Leiden.

**Results.** Eight studies were analyzed for the estimation of the risk of VTE. A total of 338 out of 2,696 cases (12.5%; range 7.8 to 18.5%) and 885 out of 7,710 controls (11.5%; range 8.1 to 12.1%) were HR2 positive. The odds ratio for VTE associated with HR2 haplotype was not statistically significant (OR 1.15; 95% C.I. 0.98-1.36). The OR for the association between FV Leiden and FV HR2 and the risk of VTE in cases and controls was largely heterogeneous as to OR and 95% C.I. and no statistical significant difference was observed.

**Interpretation and Conclusions.** The data from the present meta-analysis suggests that FVHR2 could be a very mild prothrombotic factor. The association of FV Leiden and HR2 haplotype seems not to increase significantly the risk of VTE carried by isolated heterozygosity for FV Leiden. However, well-designed clinical studies are needed to clarify this issue definitely.

**Key words:** factor V, factor V Leiden, factor V HR2, venous thromboembolism.

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Factor V (FV) is a plasma glycoprotein with a pivotal role in blood coagulation.<sup>1</sup> After proteolytic activation by thrombin or activated FX, activated FV (FVa) binds to FXa becoming a co-factor in the prothrombinase complex, which leads to the conversion of prothrombin to thrombin.<sup>1</sup> FVa is inactivated by activated protein C (APC), which thus modulates the amount of thrombin generated. Furthermore, along with protein S, FV is a co-factor of APC-mediated inactivation of FVIII.<sup>2</sup>

A common polymorphism (FV Leiden) in the FV gene that causes a missense mutation, FV Arg506Gln, results in the loss of one activated protein C cleavage site of FV.<sup>3</sup> The resulting APC resistance phenotype carries a significant risk of venous thromboembolism (VTE).<sup>4,5</sup>

Recently, a complex haplotype of FV (HR2), which includes 13 different polymorphisms throughout the gene, has been reported.<sup>6</sup> Seven of the 13 base changes predict an amino acid change in FV, and lead to functional modifications of the protein. FVHR2 has decreased co-factor activity for APC-mediated degradation of FVIIIa,<sup>7</sup> and an increased ratio of factor V1 to factor V2, the former being the more procoagulant isoform.<sup>8</sup> Increased resistance to APC and reduced FV antigen and/or coagulant activity have been associated with FVHR2,<sup>9-11</sup> though not consistently.<sup>9,12</sup> The HR2 haplotype is rather frequent, with an allele frequency throughout Asia, Europe and in native African populations ranging from 5 to 17%.<sup>9-16</sup> The very high prevalence (up to 50%) in Indian tribes from Costa Rica is notable.<sup>17</sup>

Several studies have provided contrasting results on whether the haplotype, in itself, induces a significant risk of VTE and no definite conclusions have been drawn.<sup>10-16,18</sup> However, it has been surmised that FVHR2 enhances the risk of VTE conferred by FV Leiden, since this effect was observed in a case-control study,<sup>11</sup> a study on families with FV Leiden<sup>19</sup> and in a prospective study enrolling a small number of double heterozygotes.<sup>20</sup> The mechanisms by which HR2 could increase the risk conferred by FV Leiden are probably several. First of all, both the polymorphisms reduce APC co-factor activity. Furthermore, and most importantly, FV Leiden and FV HR2 never reside on the same allele<sup>6,9</sup> and thus individuals with both defects do not have any normal factor V. The association of FV Leiden and HR2 is, however, rare, being approximately 3 in 1000 individuals in the general population and 3 in 100 unselected patients with thrombosis.<sup>9</sup> It is therefore unlikely that a single study could adequately dissect the risk of

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**Table 1. Number and type of recruitment for cases and controls.**

Authors	Cases, N.	Population	Controls, N.	Population
Alhenc-Gelas <i>et al.</i> <sup>10</sup>	205	Patients with VTE aged < 61 years, enrolled between 11/1995 to 11/1997	394	Healthy subjects aged 20-60 years, recruited during routine check-up
Akar <i>et al.</i> <sup>13</sup>	148	Patients with VTE	84	Healthy unrelated individuals
Benson <i>et al.</i> <sup>14</sup>	210	Caucasian adult patients self-reporting history of VTE from the normal population described beside	5,060	Adults subjects from a large managed-care population in Southern California
De Visser <i>et al.</i> <sup>11</sup>	471	Consecutive outpatients, aged < 70 years, with a first DVT objectively confirmed	472	Age- and sex-matched population controls
Folsom <i>et al.</i> <sup>20</sup>	301	Incident cases of VTE arising from a cohort of 20,993 subjects prospectively followed, aged > 45 years	623	Age, sex and race matched individuals from the same cohort without VTE
Kostka <i>et al.</i> <sup>16</sup>	347	Consecutive patients aged 12-92 years recruited from 1997 to 2000	282	Healthy volunteers aged 18-78 years from Dresden area
Luddington <i>et al.</i> <sup>12</sup>	581	Consecutive patients with history of VTE, aged 18-65 years	469	Blood donors aged 18-65 years, same geographical area
Margaglione <i>et al.</i> <sup>18</sup>	433	Patients with DVT aged 9-85 years enrolled between 5/1996 to 12/1999	326	Healthy hospital employees
Total	2,696		7,710	

VTE: venous thromboembolism; DVT: deep vein thrombosis.

venous thromboembolism due to the simultaneous presence of these two FV polymorphisms.

With this as a background, we decided to carry out a meta-analysis of the published studies on the VTE risk conferred by the HR2 haplotype, either alone or together with FV Leiden.

## Design and Methods

We retrieved all published reports on the possible effect of HR2 haplotype in favoring venous thromboembolism, with an extensive MEDLINE search up to March 2003. For the analysis of this risk, case-control or prospective studies reporting the prevalence of HR2 haplotype in cases and controls were selected. Likewise, the influence on VTE risk exerted by the concomitant presence of FV Leiden and HR2 was evaluated in the same studies whenever possible, by analysis of published data or by personal communication with the Authors.

## Statistical analysis

Odds ratios and their 95% confidence intervals were calculated as the estimate of the risk of developing VTE in carriers of the given polymorphism compared to the risk in non-carriers. We tested the pooled effect of a polymorphism using both fixed

(Mantel and Haenszel), and random effect (DerSimonian and Laird) models. Because the results obtained from the different models were very similar, we report the odds ratio calculated by the Peto's assumption free method and thus the calculated odds ratio for each study do not necessarily correspond to the published odds ratio. When no events were reported for a particular subgroup in a study, a value of 0.25 was automatically attributed for the calculation of the odds ratio in that study. The number of cases included in the individual study represents the weight of each study. Statistical analyses were performed with the STATA for Windows version 8.1 statistical package,<sup>21</sup> using the *metan* command (written by M. Bradburn, J. Deeks, D. Altman and available at <http://www.stata.com/stb/stb44/sbe24>).

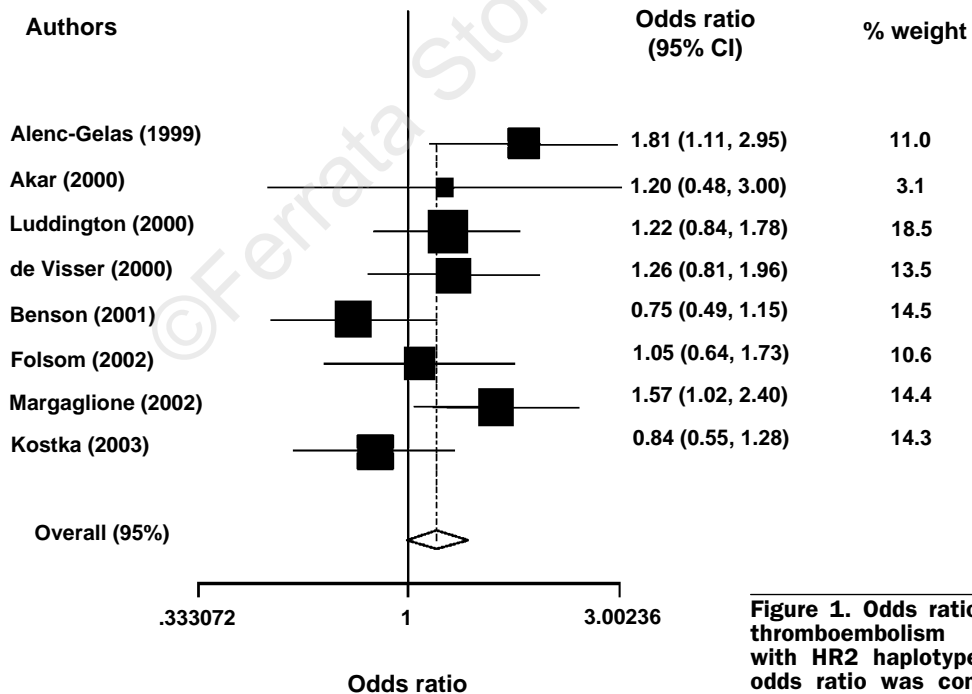
## Results

### Factor V HR2 and the risk of venous thromboembolism

Eight studies were analyzed for the estimation of the risk of VTE conferred by the HR2 haplotype (Table 1), for a total of 2,696 cases and 7,710 controls. Seven studies were retrospective; among them, only the studies by De Visser *et al.*,<sup>11</sup> Luddington *et al.*<sup>12</sup> and Kostka *et al.*<sup>16</sup> analyzed con-

**Table 2. Number and percentage of HR2 subjects in cases and controls.**

Authors	Cases, N.	HR2 positive, N. (%)	Controls, N.	HR2 positive, N. (%)
Alhenc-Gelas <i>et al.</i> <sup>10</sup>	205	38 (18.5)	394	45 (11.4)
Akar <i>et al.</i> <sup>13</sup>	148	15 (10.1)	82	7 (8.5)
Benson <i>et al.</i> <sup>14</sup>	210	19 (9.1)	5,060	611 (12.1)
De Visser <i>et al.</i> <sup>11</sup>	471	48 (10.2)	472	39 (8.3)
Folsom <i>et al.</i> <sup>20</sup>	301	26 (8.6)	623	51 (8.1)
Kostka <i>et al.</i> <sup>16</sup>	347	52 (7.8)	282	49 (8.9)
Luddington <i>et al.</i> <sup>12</sup>	581	74 (12.7)	469	50 (10.7)
Margaglione <i>et al.</i> <sup>18</sup>	433	66 (15.2)	326	33 (10.1)
Total	2,696	338 (12.5)	7,710	885 (11.5)



**Figure 1. Odds ratio for venous thromboembolism associated with HR2 haplotype. The total odds ratio was computed with Peto's method.**

**Table 3. FV Leiden and FV HR2 in cases and controls.**

Author	Cases N	FV Leiden		Controls N	FV Leiden	
		HR2-	HR2+ (%)		HR2-	HR2+ (%)
Akar <i>et al.</i> <sup>13</sup>	148	35	2 (5.7)	82	7	0
Benson <i>et al.</i> <sup>14</sup>	210	26	0	5,060	254	12 (4.7)
De Visser <i>et al.</i> <sup>11</sup>	461	75	9 (12)	472	13	1 (7.7)
Folsom <i>et al.</i> <sup>20</sup>	301	36	4 (11.1)	623	25	1 (4)
Kostka <i>et al.</i> <sup>16</sup>	347	74	11 (14.8)	282	9	2 (22.2)
Margaglione <i>et al.</i> <sup>18</sup>	433	84	5 (6)	326	19	2 (10.5)
Total	1,900	330	31 (8.6)	6,845	327	18 (5.2)

secutive cases, whereas a considerable heterogeneity of case selection was evident in the other studies. The studies by De Visser *et al.*<sup>11</sup> and Folsom *et al.*<sup>20</sup> were age- and sex-matched, with a case/control ratio of 1:1 or 1:2. Only the study by Folsom *et al.*<sup>20</sup> was prospective. The great number of controls was essentially contributed by the study of Benson *et al.*<sup>14</sup> Table 2 shows the prevalence of HR2 in cases and controls. A total of 338 cases (12.5%) and 885 controls (11.5%) were HR2 positive. The prevalence in the individual studies ranged from 7.8 to 18.5% in cases and from 8.1 to 12.1% in controls. Figure 1 shows the odds ratio for venous thromboembolism associated with HR2 haplotype in the individual studies together with the relative weight of the study contributing to the overall size of the sample. A significant effect of the haplotype was present in the studies by Alhenc-Gelas *et al.*<sup>10</sup> and Margaglione *et al.*<sup>14</sup> which together contributed about 25% of cases included in the analysis. In contrast, a possible protective effect was evident in the studies by Benson *et al.*<sup>14</sup> and Kostka *et al.*<sup>16</sup> which together contributed about 28% of cases included in the analysis. Overall, even though a possible trend was suggested, the risk was not statistically significant (OR 1.15; 95% C.I. 0.98 – 1.36;  $p = 0.082$ ).

#### **Factor V HR2/FV Leiden and the risk of venous thromboembolism**

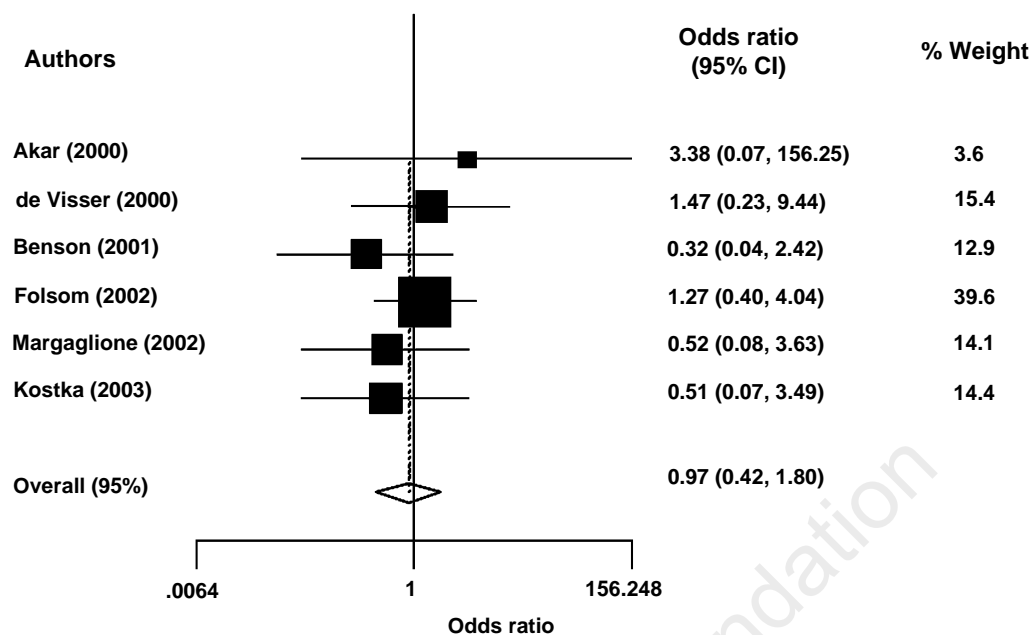
It was possible to estimate the odds ratio for the association between FV Leiden and FV HR2 only in six out of the above eight reported studies. Table 3 shows the prevalence of HR2 haplotype in cases and controls grouped according to carriership of FV Leiden. A total of 1,900 cases and 6,845 controls were available for analysis. The relative frequency

of HR2 in FV Leiden carriers was 31/361 (8.6%) in cases and 18/345 (5.2%) in controls. Figure 2 shows the odds ratio for venous thromboembolism associated with HR2 in FV Leiden carriers. The estimates were largely heterogeneous as concerns OR and 95% CI, ranging from a protective role of HR2 against thrombosis in the studies by Benson *et al.*<sup>14</sup> Kostka *et al.*<sup>16</sup> and Margaglione *et al.*<sup>18</sup> to a three-fold increased risk in the study by Akar *et al.*<sup>13</sup> this last having a very small relative weight (3.6%). However, no statistically significant effect of HR2 was observed either in a single study or in the pooled analysis, and the overall OR was below 1 (0.87, 95% CI 0.42–1.80) but substantially within the previous risk estimate for HR2 in all subjects.

Finally, we analyzed the thrombotic risk in carriers of both polymorphisms and compared this with the risk in normal subjects (FV Leiden and HR2 negative). The risk conferred by FV Leiden alone was 4.17, with a very narrow 95% CI (3.42 – 5.07), in agreement with the well recognized risk factor magnitude reported in several studies (Figure 3 A). When the analysis was restricted to FV Leiden carriers who were heterozygous for the HR2 haplotype (Figure 3 B), the magnitude of the risk did not change substantially (OR: 3.78), as roughly expected by multiplying the OR for FV Leiden with that for HR2 ( $4.17 \times 0.87 = 3.6$ ).

#### **Discussion**

The HR2 haplotype in the FV gene was first described in 1996 and its association with low FV levels in plasma was surmised.<sup>6</sup> Soon thereafter, it was demonstrated that the haplotype was also associated with a mild increase of APC resistance in the absence of FV Leiden mutation.<sup>9</sup> The question arose



**Figure 2. Odds ratio for venous thromboembolism associated with HR2 haplotype in FV Leiden carriers. The total odds ratio was computed with Peto's method.**

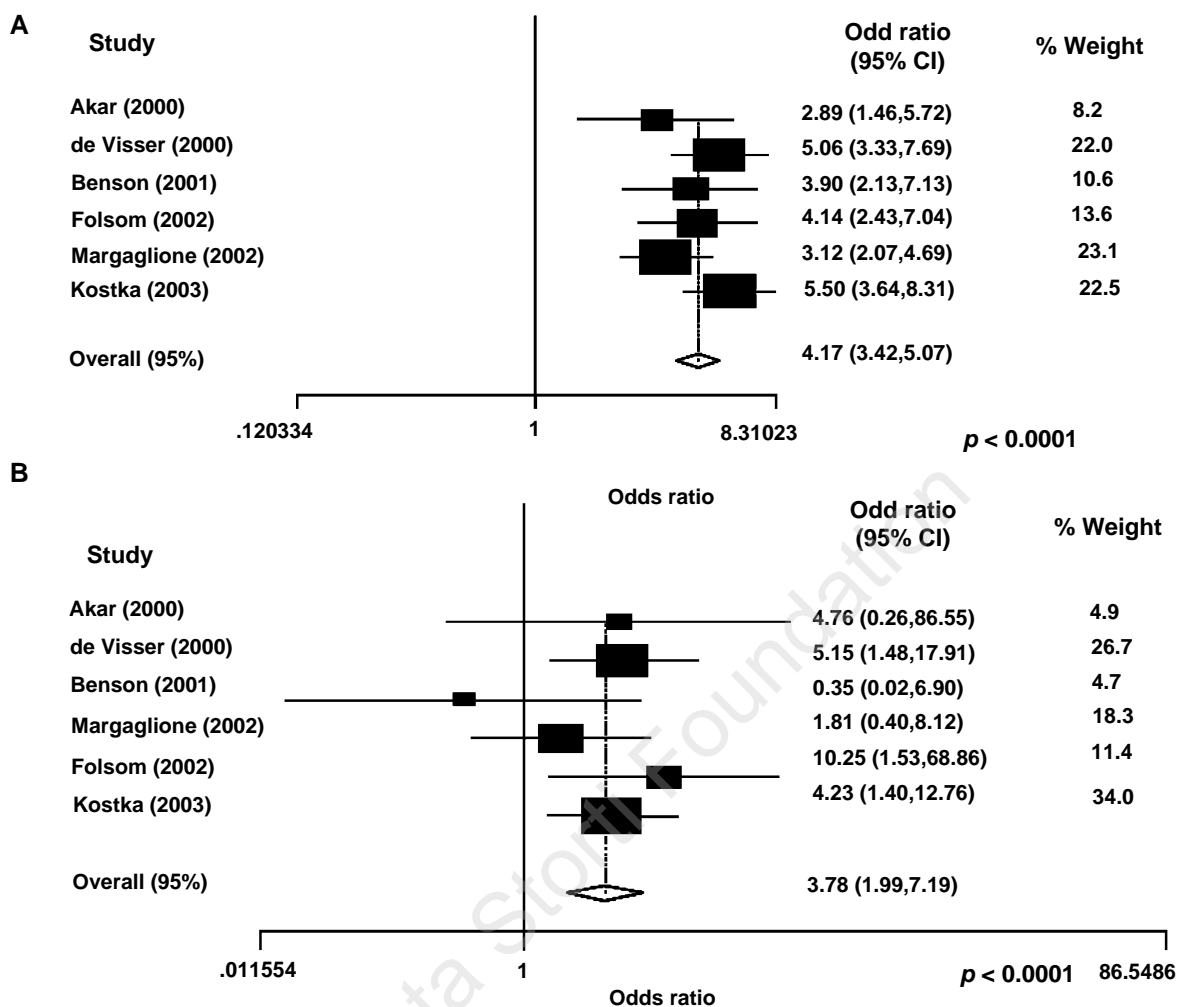
of whether the haplotype could represent a risk factor for venous thrombosis. This aspect was felt worthy of investigation since the HR2 haplotype is frequent, being found in approximately 5-10% of the studied populations, and therefore potentially relevant in the evaluation of the thrombophilic patient. Furthermore, several biochemical studies showed that HR2 possesses the potential to be a prothrombotic factor.<sup>2</sup> It has been shown that the resistance induced by Factor V HR2 is related to the inability to inactivate factor VIII (decreased cofactor activity of HR2) rather than factor V.<sup>7</sup> Thus, the slightly more enhanced resistance to APC in HR2 carriers might be related to slower factor VIII inactivation. An additional aspect to investigate was the possibility that the interaction of the haplotype with FV Leiden could increase the risk of venous thrombosis associated with the latter polymorphism.

Unfortunately, all available studies were not designed to study the interaction between these polymorphisms, and consequently are all underpowered from a statistical point of view. We, therefore, carried out a systematic review of the literature and performed a meta-analysis of the studies investigating these two aspects. As a general comment, a wide heterogeneity in study design and case/control ratio was observed in the different studies. This could be a partial cause of negative results when investigating a thrombophilic trait

associated with a mild increase of risk of venous thrombosis.

As to the role of FVHR2 in inducing venous thrombosis, a total of 2,696 cases and 7,710 controls were pooled (Table 1). The odds ratio in each study was always below 2 and only in the study by Alhenc-Gelas *et al.* was a statistically significant effect demonstrated.<sup>10</sup> In our meta-analysis, the cumulative odds ratio showed a low, not significant effect, with an odds ratio of 1.15 (95% C.I. 0.98 – 1.36;  $p=0.082$ ), which suggests that the haplotype could, at best, represent a very mild risk factor for venous thromboembolism. Further support to this is the fact that the only prospective study designed to explore the role of FV polymorphisms in venous thrombosis failed to demonstrate an association of the haplotype with this risk.<sup>20</sup> Interestingly, in that study the 3 homozygotes for HR2 all suffered from venous thrombosis, with an odds ratio of 5.5.<sup>20</sup> However, from a clinical perspective, it should be borne in mind that the frequency of homozygosity for HR2 haplotype is in the general population can usually be expected to be lower than 0.5%,<sup>9</sup> apart from in ethnically isolated exceptions.<sup>17</sup> Additional data are required before considering HR2 homozygotes at increased risk of venous thromboembolism.

The second aim of the present meta-analysis was to examine the risk of venous thromboembolism



**Figure 3. A) Odds ratio for venous thromboembolism associated with FV Leiden. B) Odds ratio for venous thromboembolism associated with compound heterozygosity for HR2 haplotype and FV Leiden. The total odds ratio was computed with Peto's method.**

exerted by the co-inheritance of FV Leiden and HR2 haplotype. The two polymorphisms never reside on the same allele<sup>9</sup> so that it is expected that co-inheritance should enhance the risk of venous thrombosis significantly compared to the risk conferred by FV Leiden alone. In support of this hypothesis, APC resistance is increased in double heterozygotes and pseudohomozygosity for APC resistance has been reported in some of these cases.<sup>22,23</sup> The first demonstration of this enhanced risk came from family studies. Faioni *et al.*<sup>19</sup> reported a relative risk of 4.2 (95% C.I. 1.6–11.3) in FV Leiden positive relatives of index cases with the mutation compared to a relative risk of 10.9 (95% C.I. 2.9 – 40.6) associated with compound heterozygosity for FV Leiden/HR2 haplotype. This figures concords

quite well with the results obtained by de Visser *et al.*<sup>11</sup> in the LETS study. In that study co-inheritance of FV Leiden and HR2 haplotype carried an odds ratio for venous thrombosis of 11.1 (95% C.I. 1.4 – 88) when compared to normal controls. However, in the LETS study only 9 patients and 1 control were compound heterozygotes, and there was a large overlap with the OR conferred by FV Leiden so that, as stated by the authors themselves, statistical uncertainty was present. Folsom *et al.*<sup>20</sup> showed an odds ratio of 16.3 (95% C.I. 1.7 – 159) in the 5/914 tested participants in their prospective study who proved to be compound heterozygotes. On the contrary, Le Cam-Duchez *et al.*<sup>24</sup> did not observe an increased risk of venous thrombosis in the 8 compound heterozygous relatives of 43 index cases with

FV Leiden mutation. Our analysis shows that the OR for venous thrombosis associated with HR2 in FV Leiden carriers were largely heterogeneous (Figure 2), ranging from the possibility of a 3-fold increased risk of venous thrombosis to a possible reduction of the risk. The pooled effect of HR2 in FV Leiden carriers (overall OR 0.87) was against its additive role and not statistically significant, with a wide 95% C.I. (0.42 – 1.80) that well comprises the previously estimated overall risk of the HR2 polymorphism in all subjects. Consequently, a supra-multiplicative effect on thrombotic risk in carriers of both polymorphisms was not observed, the risk being due mostly to FV Leiden alone (Figures 3 A and 3 B).

In conclusion, the data from the present meta-analysis suggest that FVHR2 is, at best, a very mild prothrombotic factor and the association of FV Leiden and HR2 haplotype seems not to increase the risk of venous thrombosis significantly over that conferred by isolated heterozygosity for FV Leiden. However, the published data were not able to exclude that this association could be relevant for some types of venous thrombosis (e.g., idiopathic VTE or recurrent VTE). Similarly, the thrombotic risk of the HR2 polymorphism in the homozygous state remains uncertain. At present, due to the rarity of this association, it remains strongly doubtful whether all FV Leiden carriers should also be tested for HR2. Probably, this endeavour should be tackled only in families with FV Leiden and clinical evident thrombophilia. Prospective studies, specifically designed to the purpose and with adequate size, could definitely address these topics.

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### **Pre-publication Report & Outcomes of Peer Review**

#### *Contributions*

GC and EF designed the study and wrote the manuscript; FB revised the design and the pertinent data; AT critically reviewed all the published reports and was responsible for statistical analysis. All the authors gave their final approval to the final manuscript. We thank Dr. Aaron Folsom for sharing his unpublished data.

#### *Disclosures*

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

#### *Manuscript processing*

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received March 3, 2003; accepted September 10, 2003.

In the following paragraphs, Professor Vicente summarizes the peer-review process and its outcomes.

#### *What is already known on this topic*

It has been suggested that Factor V HR2 haplotype is associated with increased resistance to APC and reduced FV plasma levels. However, the risk of thromboembolism conferred by this haplotype is controversial.

#### *What this study adds*

The data of this meta-analysis indicates that FVHR2 is a very mild prothrombotic factor. The presence of this haplotype in patients with FV Leiden seems not to increase the risk of venous thromboembolism.

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