AB0 blood group and risk of venous or arterial thrombosis in carriers of factor V Leiden or prothrombin G20210A polymorphisms

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ABSTRACT

Background

Routine analyses for thrombophilia include determination of the presence of factor V Leiden and prothrombin 20210A polymorphisms. However, the usefulness of these determinations is controversial and the clinical benefit remains questioned because of the moderate risk of associated thrombosis in carriers. In the search for clusters of thrombotic risk factors to estimate individual risk better, we studied the effect of AB0 blood group, a highly prevalent factor with mild prothrombotic features, on the risk and severity of venous and arterial thromboses in carriers of these polymorphisms.

Design and Methods

We genotyped the AB0 blood group in 981 carriers of factor V Leiden or prothrombin 20210A polymorphisms. In order to avoid the over-representation of a particular genotype and to suppress confounding factors, we included only non-related heterozygous carriers without additional genetic risk factors. We studied 609 patients with venous thromboembolism (287 with factor V Leiden, and 322 with prothrombin 20210A), 174 patients with myocardial infarction (78 with factor V Leiden, and 96 with prothrombin 20210A), and 198 controls (96 with factor V Leiden, and 102 with prothrombin 20210A).

Results

Non-OO blood group did not increase the risk of myocardial infarction in carriers of factor V Leiden or prothrombin 20210A. However, non-OO blood group contributed significantly to the expression of venous thrombosis associated with both factor V Leiden (OR: 1.76; 95%CI: 1.06-2.91) and prothrombin 20210A (OR: 2.17; 95%CI: 1.33-3.53). Exclusion of A_2A_2 and A_2O from the non-OO blood group (because factor VIII-von Willebrand factor levels are similar in these and the OO blood group) increased the thrombotic risk. Finally, non-OO blood group was associated with an earlier onset in symptomatic carriers of these polymorphisms.

Conclusions

Our study suggests that non-OO blood group increases the risk and severity of venous thrombosis in carriers of prothrombotic polymorphisms. Thus, AB0 phenotyping or genotyping analyses may be valuable components in assessing future thrombophilic risk profiles and might have implications for the policy of thrombosis prophylaxis and treatment.

Key words: AB0 blood group, factor V Leiden, prothrombin 20210A, thrombophilia, thrombosis.

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Introduction

The large proportion of patients with venous thromboembolism (VTE) carrying factor V Leiden (FVL) or the prothrombin 20210A allele (PT) explains why these polymorphisms have been included worldwide in routine analyses of thrombophilia.[1] However, the usefulness of their determination is controversial and the clinical benefit remains questioned. The identification of asymptomatic subjects carrying genetic anomalies that increase the risk of a clinically relevant disorder such as VTE may have serious ethical implications. Moreover, there is considerable uncertainty as to how this information should be used in the optimal management of carriers in terms of both the need for and the effectiveness of antithrombotic interventions.[2] The reason for such uncertainty is the relatively low risk of VTE associated with these polymorphisms, and the knowledge that VTE is a polygenic and multifactorial disease. Thus, these polymorphisms should be regarded as risk factors for thrombosis, whose clinical expression generally depends on the coexistence of additional thrombophilic mutations or environmental conditions that provoke the development of VTE. Examples of the effects of gene-gene interactions in VTE are the higher risk and greater severity of thrombosis in homozygous carriers, or subjects with combinations of distinct gene defects.[1]

These common genetic variants show a very mild association with arterial thrombotic diseases (1.17 for FVL and 1.31 for PT, according to a recent meta-analysis including 66155 cases and 91307 controls).[3] These results probably reflect the very complex nature of these disorders, in which the effect of a single functional polymorphism is too small to be consistently related to risk.

In this framework, it seems appropriate to evaluate the relevance of gene-gene interactions on thrombotic risk. Unfortunately, few studies have addressed such a possibility, particularly in arterial thrombosis.[4,5]

Numerous studies have clearly demonstrated a clinically important effect of AB0 blood group on *in vivo* hemostasis. Up to 20 studies consistently demonstrated that individuals with non-00 blood group have a significantly increased risk (ranging from 1.8 to 2.5-fold) of VTE.[6-25] An association between AB0 group and risks of ischemic heart disease and peripheral vascular disease has also been recognized.[26] In contrast, the 00 blood group is overrepresented among patients with bleeding disorders.[27-29] Interestingly, non-00 blood group seems to significantly increase the risk of VTE in carriers of FVL.[24,25,30-33] Unfortunately, these conclusions were drawn from studies with limited statistical power because of the small numbers of carriers, particularly among controls.

The objective of this study was to examine the role of the AB0 blood group on the risk of arterial and venous thromboses in carriers of FVL or PT.

Design and Methods

Subjects and genotyping

To analyze the role of the AB0 blood group as a genetic modulator of the thrombotic risk associated with two prothrombotic polymorphisms, FVL and PT, a multicenter collaborative case-control study was performed. Six Spanish hospitals participated in the study. In order to reduce the interference from other factors, only unrelated subjects were included and they had to have only a single (known) prothrombotic defect. Thus, individuals homozygous or double heterozygous for FVL or PT, who have a strong thrombotic risk, were not recruited. Similarly, patients with any of these polymorphisms and a strong thrombotic risk factor, such as known malignant disorders, antithrombin, protein C or protein S deficiency, or antiphospholipid antibodies, were also excluded.

Patients were recruited from a large series of consecutive patients with VTE or myocardial infarction who fulfilled these requirements from the six Spanish hospitals. In brief, we recruited 783 Caucasian patients: 609 with VTE (287 heterozygous carriers of FVL, and 322 heterozygous carriers of PT) and 174 with myocardial infarction (78 heterozygous carriers of FVL, and 96 heterozygous carriers of PT). Venous thrombotic events were confirmed by objective methods such as venography or compression ultrasonography for deep venous thrombosis and ventilation-perfusion lung scan for pulmonary embolism. The diagnosis of myocardial infarction required the presence of at least two of the following criteria: typical chest pain lasting more than 30 minutes; creatine kinase elevation exceeding twice the upper normal limit with a concomitant rise in the level of the creatine kinase MB isoenzyme; presence of new Q waves or new or presumed new abnormal ST-T features. We also recruited 198 unrelated Caucasian control subjects (96 heterozygous carriers of FVL, and 102 heterozygous carriers of PT) who came from the same regions as the patients. These control subjects were mainly blood donors and had neither a documented history of vascular disease, nor a personal history of arterial or venous thrombotic disease.

Patients and controls were fully informed of the aim of this study. All subjects included gave their informed consent to participation in the study, which was performed according to the declaration of Helsinki, as amended in Edinburgh in 2000. The study was approved by the Ethics Committee of the hospitals involved.

Genetic identification of FVL, PT and AB0 blood group was performed essentially as described elsewhere.[34,35]

Statistical analysis

Continuous variables are expressed as means ± standard deviations, and as percentages for categorical variables. Comparisons between groups were done using an unpaired, Student's-t test or a χ^2 test, as appropriate.

Univariate statistical analysis was performed with the χ^2 test. The strength of the association of major risk factors and the combinations of polymorphisms with the occurrence of disease was estimated using 2x2 tables and calculating the odds ratio (OR) with EpiInfo software (Centre for Disease Control, Atlanta, Georgia, USA) and the Cornfield method for the calculation of 95% confidence intervals (CI). Multivariate analysis was performed using multiple logistic regression (Enter method), with the SPSS statistical package for Windows 11.0 software (Chicago, Illinois, USA). Differences with a two-tailed p value <0.05 were considered statistically significant.

Results

Distribution of ABO genotypes

Table 1 shows the clinical features of patients and controls in all the groups studied. The method used for blood group genotyping discriminates A_1, A_2, O_1, O_2, and B alleles.[35] We identified all possible genotypes except the O_2O_2 genotype. The distribution of ABO genotypes and alleles in the groups studied are shown in Table 2. The distribution of ABO genotypes observed in controls was similar to that described in the Spanish population.[22]

Risk of myocardial infarction

The ABO genotype did not modify the risk of myocardial infarction in carriers of the FVL or PT polymorphisms. Thus, none of the non-00 genotypes (including or excluding A_2 homozygotes or A_2-O combinations) was associated with an increased risk of myocardial infarction when compared with the risk in individuals with 00 genotypes (Table 3). Multivariate analysis including classical cardiovascular risk factors confirmed the low relevance that the combination of non-00 genotypes with FVL or PT polymorphisms has in such a complex disease (Table 3).

Risk of VTE

Our results confirm previous studies that found an increased risk of VTE among carriers of FVL who had a non-O blood group.[24,25,30-33] Thus, the prevalence of non-00 genotypes was significantly higher in symptomatic carriers of FVL (71.1%) than in asymptomatic ones (58.3%) (p=0.021; OR: 1.76; 95% CI: 1.06-2.91) (Table 3). Exclusion of A_2 homozygotes or A_2-O combinations from the non-00 blood group increased the differences between patients and controls (Table 3).

Interestingly, we observed a similar effect on the risk of venous thrombosis when the non-00 blood group was combined with the PT polymorphism. Indeed, non-00 blood group increased the risk of VTE in heterozygous carriers of the PT polymorphism by 2.17-fold; when excluding A_2 homozygotes or A_2-O combinations from the non-00 group, the risk was increased yet further (2.85-fold) (Table 3).

Finally, among symptomatic patients with VTE, a non-00 blood group was associated with younger age at the onset of thromboembolism (FVL carriers:

Table 1. Clinical features of subjects included in the study.

Group	FV Leiden			PT 20210A		
	VTE	Myocardial infarction	Control	VTE	Myocardial infarction	Control
Number	287	78	96	322	96	102
Age: mean±SD	45.9± 16.9[a]	55.8± 16.9[a,b]	45.6± 15.1	46.3± 15.5[a]	51.8± 17.0[a,b]	45.7±1 4.2
Male sex (%)	137 (47.7%)	53 (67.9%)[b]	39 (40.6%)	161 (50.0%)	66 (68.8%)[b]	56 (54.9%)
Hyperlipidemia(%)	ND	29 (37.2%)[b]	13 (13.5%)	ND	47 (49.0%)[b]	14 (13.7%)
Hypertension (%)	ND	28 (35.9%)[b]	15 (15.6%)	ND	36 (37.5%)[b]	11 (10.8%)
Diabetes (%)	ND	35 (44.9%)[b]	3 (3.1%)	ND	28 (29.2%)[b]	4 (3.9%)
Smoker (%)	ND	30 (38.5%)	24 (25.0%)	ND	45 (46.9%)[b]	27 (26.5%)

[a]*Age of the first thrombotic episode for VTE and myocardial infarction patients.*
[b]*p<0.05 vs. respective control group; ND: not determined.*

Table 2. Distribution of ABO genotypes and alleles.

	FV Leiden			PT 20210A		
	VTE N=287	Myocardial infarction N=78	Control N=96	VTE N=322	Myocardial infarction N=96	Control N=102
Genotype						
A1A1	23 (8.0%)	4 (5.1%)	3 (3.1%)	44 (13.7%)	9 (9.4%)	9 (8.8%)
A1A2	9 (3.1%)	4 (5.1%)	0	15 (4.7%)	2 (2.1%)	1 (1.0%)
A101	103 (35.9%)	24 (30.8%)	32 (33.3%)	101 (31.4%)	21 (21.9%)	26 (25.5%)
A102	2 (0.7%)	1 (1.3%)	0	6 (1.9%)	4 (4.2%)	0
A1B	15 (5.2%)	5 (6.4%)	1 (1.0%)	20 (6.2%)	4 (4.2%)	4 (3.9%)
B01	34 (11.8%)	6 (7.7%)	7 (7.3%)	29 (9.0%)	8 (8.3%)	3 (2.9%)
B02	1 (0.3%)	1 (1.3%)	1 (1.0%)	4 (1.2%)	1 (1.0%)	0
BB	4 (1.4%)	0	1 (1.0%)	2 (0.6%)	1 (1.0%)	2 (2.0%)
A2B	1 (0.3%)	0	2 (2.1%)	2 (0.6%)	0	0
A201	12 (4.2%)	6 (7.7%)	8 (8.3%)	11 (3.4%)	4 (4.2%)	11 (10.8%)
A202	0	0	1 (1.0%)	2 (0.6%)	1 (1.0%)	0
A2A2	0	0	0	0	0	1 (1.0%)
0101	74 (25.8%)	24 (30.8%)	32 (33.3%)	69 (21.4%)	35 (36.5%)	38 (37.3%)
0102	9 (3.1%)	3 (3.8%)	8 (8.3%)	17 (5.3%)	6 (6.2%)	7 (6.9%)
0202	0	0	0	0	0	0
Allele						
A1	175 (0.305)	42 (0.269)	39 (0.203)	230 (0.357)	49 (0.255)	49 (0.240)
B	59 (0.103)	12 (0.077)	13 (0.068)	59 (0.091)	15 (0.078)	11 (0.054)
A2	22 (0.038)	10 (0.064)	11 (0.057)	30 (0.047)	7 (0.036)	14 (0.069)
01	306 (0.533)	87 (0.558)	119 (0.620)	296 (0.460)	109 (0.568)	123 (0.603)
02	12 (0.021)	5 (0.032)	10 (0.052)	29 (0.045)	12 (0.063)	7 (0.034)

47.7±18.3 years in 00 group patients *vs*. 45.2±16.4 years in non-00 group patients; p=0.254; PT carriers: 50.0±15.9 years in 00 group patients *vs.* 45.0±15.2 years in non-00 group patients, p=0.011).

Discussion

Numerous studies have reported significant associations between different AB0 blood groups and a variety of diverse, and occasionally bizarre, phenotypic characteristics (including higher intelligence in group A_2 individuals, increased criminality in group B individuals, and more severe hangovers in group A individuals).[36,37] Many of these early association studies reached conflicting conclusions, and interpretation of their results was further compromised by the small number of subjects involved. Consequently, for many years all reported associations with AB0 group were viewed with some scepticism. This may explain why, despite an unequivocal role of the AB0 blood group in VTE,[6-25] this prothrombotic genetic risk factor is not usually studied in analyses of thrombophilia. However, the moderate thrombotic risk associated with non-00 blood group (OR: 1.8-2.5), and the high prevalence in the general population of prothrombotic non-00 genotypes (>50%), make the AB0 blood group of general interest in thrombosis, particularly considering thrombosis as a multigenic and complex disease.[38] Complex traits can be understood by assuming an interaction between different mutations in candidate susceptibility genes. In this framework, the prothrombotic AB0 genotypes might increase the risk of both venous and arterial thromboses in carriers of other prothrombotic risk factors.

The design of our study does not enable additive or synergistic associations to be determined and we can only evaluate the risk associated with blood group in carriers of prothrombotic polymorphisms. However, this information is valuable, as our study includes a relevant number of carriers (patients and controls) of these prothrombotic polymorphisms. Moreover, exclusion of related family members avoided the over-representation of particular genotypes. We also excluded symptomatic carriers with additional risk factors from the study in order to minimize confounding factors.

Our results suggest that the combination of non-00 blood group with FVL or PT does not significantly increase the risk of myocardial infarction, supporting the concept that this is a very complex disorder in which the risk associated with mild prothrombotic genetic defects, such as FVL, PT or AB0 blood group, even in combination, is low. In contrast, our results confirm the significant contribution of non-00 blood group to the expression of VTE associated with FVL (Table 4). Furthermore, this is the first report suggesting that non-00 blood group also significantly increases the risk of VTE in carriers of PT, in contrast to two small studies that found no effect.[25,30]

There is increasing evidence that elevated factor VIII -von Willebrand factor levels may represent an important risk factor for VTE.[39] Thus, the higher concentrations of von Willebrand factor and factor VIII present in subjects with non-00 blood group[40] could explain the risk of VTE associated with these blood groups, and their effect of increasing the risk of venous thrombosis in carriers of FVL or PT. In agreement, exclusion of A_2O and A_2A_2 genotypes from the non-00 blood group (A_2 allele has been associated with lower levels of factor VIII and von Willebrand factor, similar to those observed in 00 subjects)[20,22] further increases the risk of VTE in both FVL and PT carriers. The mechanism by which non-00 blood group increases the risk of thrombosis in carriers of FVL was explained by the effect of these two factors on the activated protein C pathway. High factor VIII levels are associated with decreased responsiveness to activated protein C,[41] which is further impaired by FVL.[24] Moreover, FVL is a defective co-factor in the inactivation of activated factor VIII by activated protein C.[42] All these effects result in a more pronounced activated protein C resistance. The mechanism is similar to the small additional effect of oral

Table 3. Effect of AB0 blood group on thrombotic risk in carriers of prothrombotic polymorphisms.

Group	*FV Leiden* *VTE* *N=287*	*Myocardial infarction* *N=78*	*Control* *N=96*	*PT 20210A* *VTE* *N=322*	*Myocardial infarction* *N=96*	*Control* *N=102*
00	83 (28.9%)	27 (34.6%)	40 (41.7%)	86 (26.7%)	41 (42.7%)	45 (44.1%)
Non-00	204 (71.1%)	51 (65.4%)	56 (58.3%)	236 (73.3%)	55 (57.3%)	57 (55.9%)
Crude analysis	p=0.021	p=0.342		p=0.001	p=0.842	
OR (95% CI)	1.76 (1.06-2.91)	1.35 (0.69-2.63)		2.17 (1.33-3.53)	1.06 (0.58-1.93)	
Multivariate analysis		p=1.000		p=0.877		
OR (95% CI)		1.00 (0.47-2.13)		0.95 (0.47-1.91)		
00 + A_2	95 (33.1%)	33 (42.3%)	49 (51.0%)	99 (30.7%)	46 (47.9%)	57 (55.9%)
Non-00 – A_2	192 (66.9%)	45 (57.7%)	47 (49.0%)	223 (69.3%)	50 (52.1%)	45 (44.1%)
Crude analysis	p=0.002	p=0.251		p< 0.001	p=0.262	
OR (95% CI)	2.11 (1.28-3.46)	1.42 (0.75-2.72)		2.85 (1.76-4.62)	1.38 (0.76-2.51)	
Multivariate analysis		p=0.386			p=0.374	
OR (95% CI)		1.39 (0.66-2.93)			1.37 (0.68-2.76)	

OO group includes O_1O_1; O_1O_2 and O_2O_2 genotypes. Non-OO group includes A_1A_1, A_1A_2, A_2A_2, A_1O_1, A_1O_2, A_2O_1, A_2O_2, A_1B, A_2B, BO_1, BO_2, and BB genotypes. A_2 group includes A_2O_1, A_2O_2 and A_2A_2 genotypes. Covariates in multivariate analysis for myocardial infarction include age, sex, and cardiovascular risk factors.

Table 4. Influence of the non-OO blood group on the risk of venous thrombosis in heterozygous carriers of factor V Leiden.

Study (reference)	*Patients*	*Controls*	*Odds*
This study	287	96	1.8
Morelli *et al.* [24]	92	14	5.1
Ohira *et al.* [25]	51	32	2.6
Robert *et al.* [31]	137	32	3.9
Hiltunen *et al.* [33]	7	14	4.5

contraceptive use on the activated protein C sensitivity ratio in FVL carriers, which also increases the risk of thrombosis.[43] However, as suggested elsewhere, AB0 blood group may have additional consequences on other factors that might also contribute to the risk of thrombosis,[24,25] and deserve additional investigation, particularly to explain the higher thrombotic risk observed in carriers of PT.

These results suggest that non-00 blood group, probably by association with higher factor VIII-von Willebrand factor levels, significantly increases the risk of VTE, and contributes substantially to the incidence of VTE in carriers of FVL or PT. The impact of one prothrombotic factor differs according to the baseline risk of the population. It would, therefore, be interesting to study the contribution of these combinations in specific populations (such as in the elderly). Finally, it would be valuable to determine the role of non-00 blood group in the risk of venous thrombosis when combined with other risk factors, whether genetic (deficiencies of antithrombin, protein C or protein S) or acquired (surgery, immobilization, pregnancy, oral contraceptives).

VTE is a multicausal disease in which several risk factors, both genetic and acquired, have to occur simultaneously to cause thrombosis. Thus, most individuals with a single thrombophilic risk factor (particularly with common prothrombotic polymorphisms such as FVL and PT) are asymptomatic. Moreover, the risk of recurrence or the duration of anticoagulant therapy is generally not altered by thrombophilia. Therefore, in the vast majority of cases, genetic testing for thrombophilia is of limited value to the symptomatic patient, and provides minimal benefit over and above the family history, and yet raises serious ethical concerns related to insurance and social security issues in asymptomatic carriers of single thrombophilic defects.[2,44] Accordingly, it is necessary to identify clusters of risk factors to estimate an individual's risk of thromboembolic events better. Our results suggest that non-00 blood group significantly increases the risk of VTE in carriers of FVL or PT. This and the high prevalence in Caucasian population of the non-00 blood group indicate that AB0 phenotyping or genotyping analyses may be valuable components in assessing future thrombophilic risk profile and might have implications for the policy of thrombosis prophylaxis and treatment.

Authorship and Disclosures

AM, AO: experimental work, collection, organization, and analysis of all data and review of the manuscript; JC, VV: project planning, design of the study, experimental work, data interpretation and analysis, manuscript writing; FE, JRG-P, RL, JF, PL, FM, AE and IA: collection of clinical data, experimental work, analysis of data, review of the manuscript. All authors approved the final version of the manuscript. The authors reported no potential conflicts of interest.

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