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Review

Phenotypic Heterogeneity in Patients with Homozygous Prothrombin 20210AA Genotype

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Venous thromboembolic events (VTEs) affect an estimated 1 in 1000 people annually, resulting in ~50,000 deaths, with prevalence increasing with age. The genetic contributors to thrombosis have been described and further explored within the last 15 years as molecular diagnostic techniques have become more widely used. The prothrombin G20210A mutation is the second most common inherited thrombotic risk factor after factor V Leiden. Generally present in less than 5% of the population, the mutation's prevalence varies greatly with ethnicity. The G20210A mutation confers a mildly increased thrombotic risk that is compounded by the presence of other risk factors. One striking characteristic of the G20210A mutation is the phenotypic heterogeneity of the rare homozygous cases. Forty percent of the reported homozygous cases are asymptomatic. Many of the symptomatic patients have additional risk factors that might compound the thrombotic risk. We present here a review of the literature for the homozygous prothrombin G20210A mutation and describe additional cases that exemplify the heterogeneous nature of this entity. ***(J Mol Diagn 2006, 8:420–425; DOI: 10.2353/jmoldx.2006.060014)***

Venous thromboembolic events (VTEs) affect an estimated 1 in 1000 people annually, resulting in ~50,000 deaths.[1] The prevalence increases with age, demonstrated by one large prospective study of men, followed from 50 to 80 years of age, with an overall incidence of VTE of 387 per 100,000 observation-years, with 107 fatal events per 100,000.[2] A variety of well-established acquired risk factors exist for these events, including recent surgery, cancer, immobility, obesity, and current or former smoking.[1,3] Use of oral contraceptives has also been identified as an acquired risk factor.[1,4] The contribution of inheritance to VTEs has long been noted but is still incompletely understood.[5,6] Although the first description of a biochemical basis for inherited thrombophilia, antithrombin deficiency, was 40 years ago, the most prevalent genetic contributors to VTE have been described and further explored only within the last 15 years as molecular diagnostic techniques have become more widely used in the effort.[6] The most prevalent hereditary risk factor, factor V Leiden, is the most common cause of resistance to activated protein C. The mutation for factor V Leiden was first described in 1994 as a single nucleotide substitution in the factor V gene G1691A.[7–9]

Prothrombin G20210A

The prothrombin gene mutation, G20210A, is the second most prevalent hereditary risk factor for VTE. Prothrombin, or factor II, is the precursor for thrombin, the vitamin K-dependent factor in the coagulation cascade that converts fibrinogen to fibrin. The prothrombin gene, located on chromosome 11 (11p11-q12), is a 21-kb gene containing 14 exons.[10] First described by Poort and colleagues in 1996,[11] the G20210A prothrombin gene mutation is a single nucleotide substitution of adenine for guanine in the 3′-untranslated region of the gene.

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Role of the Prothrombin G20210A Mutation in the Pathogenesis of Thrombophilia

The prothrombin G20210A mutation is thought to elevate prothrombotic risk through a gain of function, resulting in an increased level of prothrombin. Many studies have shown elevated prothrombin levels in patients with mutations, which are generally more pronounced in homozygous cases.[11–17] In addition to finding elevated prothrombin levels in patients with the 20210A allele, Poort and colleagues[11] found that the prothrombin level itself was a risk factor for thrombosis. The G20210A mutation has been shown to increase the efficiency of the 3′ end cleavage signal, resulting in increased cleavage site recognition, improved processing, and a more effective poly(A) site.[18,19] The end result is accumulation of mRNA and increased protein synthesis, and the resulting increase in prothrombin level is thought to confer the increased risk of thrombosis.[18,19]

Prevalence and Risk of Venous Thromboembolism

The initial Poort and colleagues[11] study found that the mutation was present in 18% of patients with a personal and family history of VTE and 6.2% of unselected patients with first time VTE, compared to 2.3% of healthy controls. Subsequent studies show prevalence in healthy European and American patients ranging from 1.2 to 4.6%.[20–25] In one large Jewish study, Zivelin and colleagues[20] found a prevalence of 6.7% of 464 Ashkenazi Jews, 5.5% of 273 Sephardic Jews, 4% of 247 Iraqi Jews, 2% of 199 Iranian Jews, 1% of 310 Yemeni Jews, and none of 177 Ethiopian Jews. The mutation is less prevalent in non-Jewish, non-European populations.[20,26–29] Dilley and colleagues[28] found the mutation in only 1 of 318 black infants (0.2%) and no mutations in an additional 185 black control patients. In their study of Brazilians, Arruda and colleagues[26] found no prothrombin G20210A mutations within their Brazilian Indian population, whereas ~2% of their cases of African descent carried the mutation. The mutation has been described in 1% of Kirghiz, 1.2% of Azerbaijani, 1.1% of Turkish, 0.3% of Indian, and 0% of other central and southeast Asian populations.[27] One Chinese study did not find the mutation in any of 1323 patients.[29] Although some studies have found no evidence that the G20210A mutation is a risk factor for VTE,[21] the general consensus is that presence of the mutation is associated with mildly increased risk. The reported odds ratios range from 1.9 to 11.5, with most falling between 2 and 4.[5,8,11,22–24,30,31]

Homozygous Prothrombin G20210A Mutation

The homozygous mutation is rare, with 67 reported cases in the literature.[11–17,21–23,26,31–57] Although more severe thrombotic risk may be expected in the homozygous state, the cases described demonstrate a broad clinical spectrum with striking heterogeneity, from a 72-year-old man who remained asymptomatic despite two surgical procedures to young patients with severe and recurrent VTEs.[36,47,50,57]

No single current review including all published cases of homozygous prothrombin G20210A mutation exists. Three published reviews of the literature, however, do provide overlapping summaries of most reported cases.[32,43,58] Girolami and colleagues[58] reviewed 18 homozygous cases and determined that many of the reported cases were described either in asymptomatic individuals (8 of 18) or in individuals with other risk factors (6 of 18). They concluded that the G20210A mutation probably represents only a mild prothrombotic state. Souto and colleagues[43] reported two asymptomatic cases and reviewed 36 cases. The age of their reviewed cases ranged from 18 to 74 years. Eleven of the twenty-eight cases with available data were asymptomatic, and 4 of the 17 symptomatic cases had additional associated risk factors such as factor V Leiden and hyperhomocysteinemia.[43] In a more recent review of 45 cases by Boinot and colleagues,[32] the heterogeneity of the cases is also evident: 22 of 45 cases were asymptomatic; the thrombotic events ranged from superficial venous thrombosis and deep venous thrombosis to pulmonary embolism, mesenteric venous thrombosis, retinal artery and vein thrombosis, and stroke; and a variety of additional risk factors was noted in a majority of the symptomatic cases.

Table 1 summarizes 49 cases, including 45 cases that have been reported either as case reports or as part of family studies, one previously reported case from William Beaumont Hospital,[57] and three additional cases from the files of the William Beaumont Hospital Department of Clinical Pathology that have not been previously reported. A review of these 49 cases illustrates the phenotypic heterogeneity typical of homozygous prothrombin G20210A mutations. The ages of these cases ranged from neonate to 74 years. Of the 49 cases, 18 (36.7%) were asymptomatic. This rate may reflect selection bias in some studies. Acquired risk factors were present in 31 of 41 cases (75.6%) for which this information was available. Family history was positive in 32 of 46 cases (69.6%) where it was reported. Additional risk factors were evaluated in 43 of the 49 case reports, and 18 of the 43 (41.9%) had additional risk factors (heterozygous MTHFR mutations not included). Factor V Leiden, the most prevalent additional risk factor in evaluated cases, was present in 10 of the 43 cases (23.3%).

Summary of Homozygous Cases at William Beaumont Hospital

Results for the prothrombin G20210A mutation analysis performed at William Beaumont Hospital from the inception of clinical testing in October 1998 to October 2005 were reviewed for cases of homozygous mutations. Of the 4570 tests performed during the period examined, there were 245 heterozygous and four homozygous results. Selection bias and lack of knowledge about the ethnic background and the reasons for clinical testing in this population prevent a meaningful comparison of the

Table 1. Prothrombin 20210 AA Homozygous Case Reports

Publication	Age/sex	Event	Acquired risk factors	Family history	Additional risk factors
Scott et al 1997[33]	18, female	DVT, ileo-femoral	Pregnancy	Negative	Negative
Howard et al 1997[41]	24, male	Myocardial infarction; subsequent DVT; PE	Smoking, surgery, and immobilization	Negative	FVL heterozygote
Kyrle et al 1998[13]	56, male	DVT, right leg; phlebitis	Not reported	Positive	Negative
	52, female	Phlebitis, bilateral legs, recurrent	Pregnancy	Positive	Negative
Gonzalez Ordonez et al 1998[35]	65, male	Thrombotic transient ischemic attacks; DVT, femoro-iliac	Surgery	Not reported	Negative
Zawadzki et al 1998[14]	48, male	DVT; PE; mesenteric venous thrombosis	Not reported	Positive	MTHFR C677T het*
	30, female	PE	Not reported	Positive	MTHFR C677T het*
Morange et al 1998[15]	44, male	DVT, left popliteal; PE	Not reported	Positive	MTHFR C677T het*
	74, female	Asymptomatic	Pregnancies	Positive	MTHFR C677T hom
	33 to 43, female (three cases)	Asymptomatic	Pregnancy, surgery	Positive	MTHFR C677T (2 hom, 1 het)*
Alatri et al 1998[36]	72, male	Asymptomatic	Surgeries	Positive	Negative
Girolami et al 1999[39]	29, male	Asymptomatic	Surgery	Negative	Not reported
	39, male	Asymptomatic	OC, pregnancies	Negative	Not reported
Girolami et al 1999[40]	21, female	Asymptomatic	Surgery	Positive	Not reported
	15, female	Asymptomatic	Negative	Positive	Not reported
Giordano et al 1999[42]	31, female	Phlebitis, left leg; TIAs; ischemic stroke	Negative	Negative	Anticardiolipin antibodies
Eikelboom et al 1999[16]	66, female	DVT, left leg	Minor surgery	Positive	Negative
	68, male	Asymptomatic	Not reported	Positive	Not reported
Souto et al 1999[43]	51, male	Asymptomatic	Negative	Positive	Negative
	19, female	Asymptomatic	Negative	Positive	Negative
Akar et al 1999[44]	73, male	Asymptomatic	Diabetes, carcinoma	Not reported	Not reported
Meinardi et al 1999[46]	34, male	DVT	Negative	Negative	FVL homozygote
Halbmayer et al 1999[50]	23, male	DVT, left popliteal; PE	Negative	Positive	FVL heterozygote
	26, female	PE	Surgery	Positive	FVL heterozygote
	20, female	Asymptomatic	Negative	Positive	Negative
Kling et al 1999[51]	44, male	Retinal vein and retinal artery occlusion	Lymphoma	Positive	Negative
Corral et al 1999[17]	45, female	DVT	Surgery	Positive	FVL heterozygote
	43, male	DVT, PE	Trauma, vascular injury	Positive	FVL heterozygote
	34, female	DVT	Pregnancy	Positive	FVL heterozygote
Bauduer et al 2000[37]	40, male	Mesenteric venous thrombosis	Obesity	Positive	Negative
Martlew et al 2000[38]	31, female	Asymptomatic	Pregnancies	Negative	MTHFR C677T het*
Acquila et al 2000[45]	22, female	DVT, left leg	Pregnancy	Negative	Negative
Sivera et al 2000[48]	28, female	DVT, femoral-iliac	OC, systemic lupus	Negative	Anticardiolipin antibodies
	2, male	Asymptomatic	Not reported	Positive	Negative
Soria et al 2000[49]	9, male	DVT, right popliteal	Negative	Negative	FVL hom, MTHFR C677T hom*
Wulf et al 1999[52]	18, male	Superficial thrombosis	Negative	Positive	FVL homozygote
	15, female	Asymptomatic	Not reported	Positive	Negative
Vaya et al 2001[56]	19, female	DVT, recurrent	OC, smoker	Unknown	Negative
Kosch et al 2002[47]	13, male	DVT, bilateral legs; PE, recurrent	Immobilization	Positive	Protein S deficiency
	19, male	Asymptomatic	Not reported	Positive	Protein S deficiency
Boinot et al 2003[32]	13, male	DVT, bilateral femoral; PE, bilateral	Immobilization	Positive	Protein C deficiency, Protein S deficiency
Kurkowska-Jastrzebska et al 2003[54]	29, female	Cerebral venous thrombosis	OC	Negative	FVL heterozygote
Klein et al 2004[53]	29, female	Eclampsia, HELLP syndrome	Pregnancy	Negative	Negative
WBH Klein et al 2004[57]	Neonate, female	Cerebral venous sinus thrombosis, PE	None	None noted	MTHFR C677T het*, low antithrombin
WBH not previously reported	33, female	DVT leg, PE	OC, former smoker	Yes, father DVT	MTHFR A1298C het*
WBH not previously reported	63, male	Recurrent DVTs arm, subsequent DVT leg	Former smoker	Yes, mother PE	MTHFR C677T het*
WBH not previously reported	43, male	DVT leg	Former smoker	None noted	Low antithrombin

*MTHFR mutations included for completeness, see text.

VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; OC, oral contraceptive use; FVL, Factor V G1691A mutation; het, heterozygous; hom, homozygous; WBH, William Beaumont Hospital cases.

prevalence data from this group to other data reported in the literature.

Our four cases confirm the heterogeneity found to date in the literature. As would be expected based on the selection of our cases from a population referred for thrombophilia testing, none of our cases were asymptomatic. The cases nonetheless varied dramatically. The ages ranged from neonate to 63 years. VTEs in the cases included deep venous thrombosis of lower and upper extremities, pulmonary embolism, and cerebral venous sinus thrombosis. Recurrent thromboembolic episodes occurred in one case. Three of four cases had acquired risk factors, including a history of smoking in three of four patients and oral contraceptive use in one patient. Two cases had decreased antithrombin levels. A positive family history of thrombosis was noted in two patients. None of the four cases carried the factor V Leiden mutation, which was the most prevalent additional risk factor described in the case literature.

Homozygous Cases Reported within Broader Studies

An additional 21 homozygous prothrombin G20210A cases have been reported and described to varying degrees as part of epidemiology studies.[11,12,21–23,26,31,34,55] The original Poort and colleagues[11] study describes a homozygous case found during familial analysis of one of the VTE probands. This case was the sister of a patient with heterozygous proband, had also experienced a VTE of unspecified type, and was additionally heterozygous for factor V Leiden. No other homozygous cases were present in the remaining proband familial analyses or in 474 Leiden Thrombophilia Study patients or their age- and sex-matched controls. In their study of multiple prothrombotic factors, Salomon and colleagues[55] describe three homozygous G20210A cases in 162 patients with VTEs, and none in 336 consecutively admitted control patients without a history of thrombotic events. In their large prospective cohort study, Ridker and colleagues[21] found no homozygous cases in 833 men that developed myocardial infarction, stroke, or venous thrombosis but did find one (asymptomatic) homozygous case in 1774 age- and smoking-status-matched male controls (0.06%). Kapur and colleagues[22] described 1 homozygous case in 21 patients with a history of venous thrombosis, and no homozygous cases in 29 patients with a history of arterial thrombosis or 50 patients without thrombosis. The homozygous case was in a 66-year-old male, with a positive family history of thrombosis, who developed retinal vein occlusion, multiple deep venous thromboses, and a pulmonary embolus throughout 4 years and who had elevated homocysteine but no other risk factors. In a recent family study of the prothrombin mutation, Bank and colleagues[31] found that the five homozygous cases in their study had an elevated risk of VTE, but these cases were not specifically further described as to the presence, type, site, or severity of VTE, or the co-existence of other risk factors.

Multifactorial Risk Profile of VTEs

The high prevalence of multiple co-existent risk factors in the reported cases of the prothrombin G20210A mutation led to ultimately disproved speculation that the prothrombin mutation was not by itself a significant risk factor and that other existing genetic and acquired risk factors are responsible for the VTEs in these cases.[59,60] Consensus within the literature now shows that the prothrombin mutation is a weak but significant risk factor for VTE. A second, crucial observation about the prevalence of coexistent risk factors is that prothrombotic risk is inextricably multifactorial, making the overall prothrombotic risk for any given individual dependent on the cumulative or synergistic effect of many different acquired and genetic factors.[32,61,62] This idea is supported by the many-fold increased risk of the prothrombin mutation or factor V Leiden mutation when combined either with each other or with oral contraceptives.[4,17,55] In their case control study of 148 women with a first episode of deep venous thrombosis and 277 healthy controls, Martinelli and colleagues[4] reported an odds ratio of 16.3 (95% CI 3.4 to 79.1) for those who had the prothrombin mutation and used oral contraceptives compared with odds ratios of 2.7 (95% CI 0.6 to 12.7) for those with the prothrombin mutation alone and 4.6 (95% CI 2.6 to 8.0) for those with a normal genotype who used oral contraceptives. Several studies have examined the combined effect of the factor V Leiden and prothrombin mutations. Zoller and colleagues[63] reported an odds ratio for thrombotic events of 11.8 (95% CI 2.6 to 53.8) for those with both mutations compared with 2.0 (95% CI 0.22 to 17.9) for the prothrombin mutation alone and 4.4 (95% CI 2.1 to 9.0) for the factor V Leiden mutation alone. Ehrenforth and colleagues[64] reported an additional threefold increased relative risk (95% CI 0.8 to 11.7) of juvenile VTE in those carrying both the factor V Leiden mutation and the prothrombin mutation, compared with those with the factor V Leiden mutation alone. Emmerich and colleagues[65] studied the combined effect of factor V Leiden and prothrombin mutations in their pooled analysis of eight case-control studies involving 2310 VTE cases and 3204 controls, and found an odds ratio of 20.0 (95% CI 11.1 to 36.1) for double heterozygotes, compared with 4.9 (95% CI 4.1 to 5.9) for factor V Leiden and 3.8 (95% CI 3.0 to 4.9) for the prothrombin mutation. Salomon and colleagues[55] studied the combined effects of prothrombotic factors in 162 patients with VTE and 336 controls, and found an odds ratio for VTE risk of 58.6 (95% CI 22.1 to 155.2) for those with both the factor V Leiden mutation and the prothrombin mutation, compared with odds ratios of 16.3 (95% CI 8.5 to 31.3) for factor V Leiden alone and 3.6 (95% CI 1.8 to 7.3) for those with the prothrombin mutation alone. Those patients that carried both mutations and were homozygous for MTHFR polymorphisms had an odds ratio of 125.8 (95% CI 38.8 to 408.0). Other studies have examined the potential additive effect of the MTHFR C677T mutation on other genetic prothrombotic risk factors with conflicting results.[24,66,67] Although no evidence exists to suggest that heterozygous C677T mutation is an independent or additive risk factor for VTE, information

about this mutation has been included in this review for the sake of completeness. The multifactorial nature of thrombotic risk underscores the importance of multiparameter testing in the appropriate clinical setting.[68,69] Prothrombin G20210A mutation analysis is now one of many tests in the recommended battery to be used when an inherited thrombophilia is suspected.[6,68]

Conclusion

The prothrombin G20210A mutation is a weak but consistent risk factor for VTEs. Homozygous G20210A mutations are rare, with 70 cases reported throughout the literature (including cases described herein). These homozygous cases display a striking phenotypic heterogeneity, from those remaining asymptomatic throughout life despite numerous other risk factors to those suffering from fatal events in the neonatal period. The current body of literature supports a multifactorial model for risk of venous thromboembolism, demonstrating that thorough clinical and laboratory evaluations must be performed and integrated to obtain an accurate picture of the overall risk for any given patient.

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