Quebec Platelet Disorder: Update on Pathogenesis, Diagnosis, and Treatment

Jessica Blavignac, B.Sc.,[1] Natalia Bunimov, Ph.D.,[1] Georges E. Rivard, M.D., F.R.C.P. (C), F.A.A.P.,[2] and Catherine P.M. Hayward, M.D., Ph.D., F.R.C.P. (C)[1]

ABSTRACT

Quebec platelet disorder (QPD) is an autosomal dominant bleeding disorder associated with reduced platelet counts and a unique gain-of-function defect in fibrinolysis due to increased expression and storage of urokinase plasminogen activator (uPA) by megakaryocytes. QPD increases risks for bleeding and its key clinical feature is delayed-onset bleeding, following surgery, dental procedures or trauma, which responds only to treatment with fibrinolytic inhibitors. The genetic cause of the disorder is a tandem duplication mutation of the uPA gene, *PLAU*, which upregulates uPA expression in megakaryocytes by an unknown mechanism. The increased platelet stores of uPA trigger plasmin-mediated degradation of QPD α-granule proteins. The gain-of-function defect in fibrinolysis is thought to be central to the pathogenesis of QPD bleeding as the activation of QPD platelets leads to release of uPA from α-granules and accelerated clot lysis. The purpose of this review is to summarize current knowledge on QPD pathogenesis and the recommended approaches to QPD diagnosis and treatment.

KEYWORDS: Quebec platelet disorder, urokinase plasminogen activator, platelets, congenital platelet disorders, fibrinolysis, congenital thrombocytopenia

Quebec platelet disorder (QPD) is a platelet disorder with a unique gain-of-function in fibrinolysis due to increased expression and storage of urokinase plasminogen activator (uPA) in platelets despite fairly normal levels of uPA in plasma and urine.[1–6] Recent studies have revealed important new information about QPD pathogenesis, including the genetic mutation which is a tandem duplication of a large region on chromosome 10 that includes the uPA gene, *PLAU*, and all of its characterized regulatory elements.[2,3,5,7] This review provides an update on the features of QPD, its pathogenesis, and the recommended treatments for this disorder which differ considerably from most platelet disorders.

HISTORY OF QPD

QPD was initially called "factor V Quebec" when Tracy and colleagues reported on an autosomal dominant bleeding disorder, affecting a large family in the province of Quebec, which was characterized by defective platelet but not plasma factor V.[8] Subsequently, the platelet-factor V abnormalities in the disorder were identified to be associated with abnormal proteolytic degradation of

[1]Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario; [2]Service d'hématologie-oncologie, CHU Sainte-Justine, Montréal, Québec, Canada.

Address for correspondence and reprint requests: Catherine P.M. Hayward, M.D., Ph.D., F.R.C.P. (C), Department of Pathology and Molecular Medicine, McMaster University, 2N29A, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada (e-mail: haywrdc@mcmaster.ca).

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multiple α-granule proteins, in addition to variably reduced platelet counts (on an average ~50% lower than the platelet counts of unaffected relatives) and abnormal platelet aggregation tests, particularly with epinephrine.[9–12] As a result of the different platelet abnormalities, the condition was redesignated as QPD.[9,10]

CLINICAL FEATURES OF QPD

Individuals affected with QPD typically have a positive personal and family history of bleeding.[13] Unlike most platelet disorders, which typically cause immediate bleeding problems with challenges, QPD bleeding becomes problematic 12 hours to 4 days after a hemostatic challenge unless treated with a fibrinolytic inhibitor.[13] Other treatments, including platelet and plasma transfusions, are not effective.[9,10] Some QPD symptoms (e.g., large bruises, nosebleeds, menorrhagia) are difficult to distinguish from those of most platelet disorders, whereas other symptoms are more suggestive of QPD as they are not typical of platelet disorders (e.g., delayed bleeding and joint bleeds).[13] As platelet counts are reduced, on an average by ~50% in QPD, only some persons with QPD have thrombocytopenia.[13]

Bleeding risks for QPD have been formally estimated by comparing the bleeding histories for affected and unaffected relatives, gathered using a standardized bleeding history tool.[13] Fig. 1 summarizes information on QPD bleeding risks, expressed as odds ratios (OR) for different bleeding problems. QPD bleeding is associated with an increased risk for bleeding that leads to changes in lifestyle.[13] However, there is variation in the disease severity (reflected by scores for bleeding symptoms) among persons with QPD, with some experiencing fairly severe problems and others experiencing minimal or no bleeding symptoms, particularly if they were treated with fibrinolytic inhibitors for all of their significant hemostatic challenges.[13] Persons with QPD have an increased risk for experiencing very large bruises or hematomas that track downward.[1,13] Approximately 50% experience joint bleeds which can lead to a destructive arthropathy.[13] Some experience delayed wound healing, which is associated with lower platelet counts.[13] Some experience episodic, spontaneous hematuria that resolves without intervention, and is associated with higher levels of platelet uPA.[13] Some bleeding complications, such as compartment syndrome, are rare but have occurred in persons with QPD who did not receive fibrinolytic inhibitor therapy.[14]Persons with QPD have similar numbers of offspring and successful pregnancies as unaffected relatives.[13] Menorrhagia is reported by some women with QPD.[13]

Although a study of the cause of deaths in persons with QPD had not been undertaken, none are known to have suffered from angina, myocardial infarction, or thrombotic stroke,[13] although some have developed peripheral vascular disease (G.E.R., unpublished observations). Additionally, some have suffered a venous thrombotic event while receiving fibrinolytic inhibitor therapy, without concurrent anticoagulation, during a period of high thrombotic risk.[13] This issue is discussed further in the section on treatment.

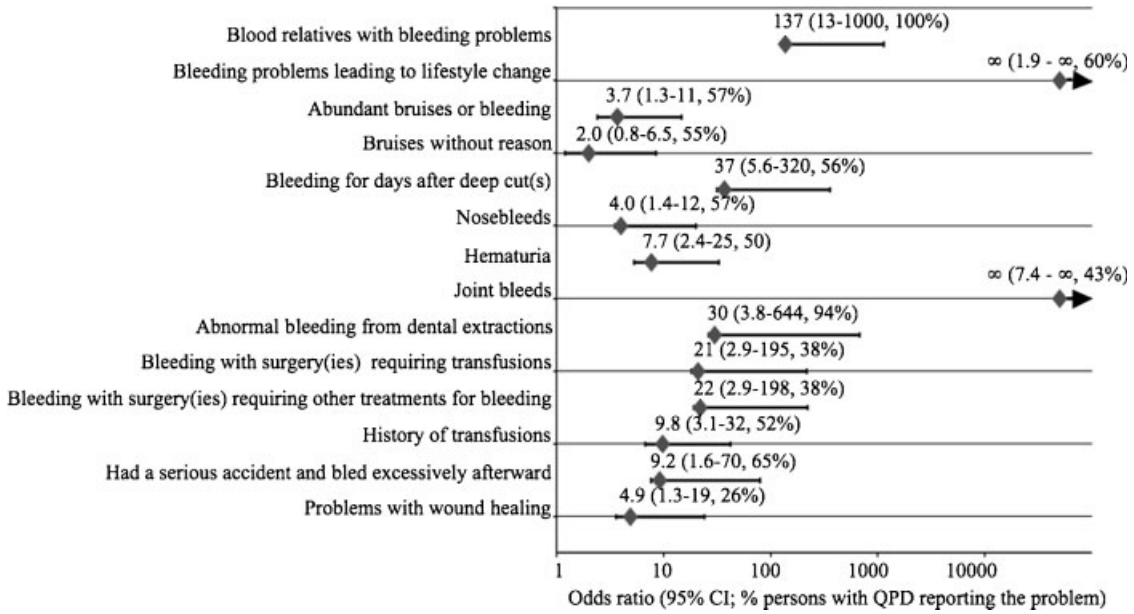

Figure 1 Bleeding manifestations associated with Quebec platelet disorder (QPD). The figure was generated from the authors' previously published data.[13] Higher odds ratio (OR) indicate a higher risk of bleeding in QPD individuals compared with unaffected relatives. CI indicates confidence interval. ∞ indicates OR estimated as infinite.

GENETIC CAUSE OF QPD

At present, there is only one known genetic cause of QPD.[3,7] The disorder is linked to a region of chromosome 10 containing *PLAU*, the uPA gene.[3] Although *PLAU* and its characterized regulatory elements have a normal sequence in QPD,[3] there is a direct, tandem duplication mutation of *PLAU* in QPD that spans a 78 kb region (Fig. 2).[7] The region duplicated encompasses all characterized regulatory elements of *PLAU* (which are located up to 2.5 kb upstream of the transcription start site and in the 3′ untranslated region), *C10orf55* (a gene of unknown function on the strand antisense to *PLAU*) and extended upstream and downstream regions (Fig. 2).[3,7,15] The breakpoint is located 57.74 kb downstream of the centromeric copy of *PLAU* and 11.87 kb upstream of the *PLAU* transcription start site for the telomeric copy of *PLAU* on the disease chromosome.[7] The duplicated segment does not include the neighboring genes that encode calcium/calmodulin-dependent protein kinase IIγ (*CAMK2G*) and vinculin (*VCL*),[7] (Fig. 2) which are not overexpressed in QPD platelets.[2]

The *PLAU* duplication mutation is specific for QPD, as it is shared by persons with confirmed QPD but it was not found among 114 unaffected relatives or 311 unrelated healthy individuals.[7]

INCIDENCE OF QPD

QPD has been diagnosed in persons living in the Canadian provinces of Quebec, British Columbia and Ontario although cases have also been diagnosed in the United States. A population-based study of QPD prevalence has not been undertaken and most cases have been diagnosed following family investigations of an index case. The authors' data on confirmed cases of QPD indicate that in Quebec, the prevalence of the disorder is ~1:220,000 individuals, and in Canada, ~1:655,000 individuals. It is possible that the actual prevalence is much higher as new cases continue to emerge through investigations of persons with bleeding problems. Most but not all cases of QPD have been traced back to a single family originally from the region of Sorel/Yamaska/St-François-du-Lac in the province of Quebec (unpublished observations). A person with impaired epinephrine aggregation from India was recently reported; however, this person was not verified to have more specific features of QPD by genetic tests or assays for increased platelet uPA and α-granule protein degradation.[16]

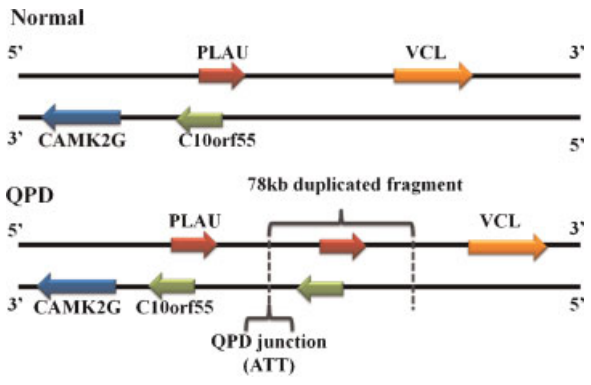

Figure 2 Diagramatic representation of the tandem duplication of *PLAU* in QPD. The duplicated region includes *PLAU*, all its characterized regulatory elements, and the uncharacterized gene *C10orf55* on the strand antisense to *PLAU*. The breakpoint has an overlap of three nucleotides (ATT) with the normal sequence. The genes calcium/calmodulin-dependent protein kinase-IIγ (*CAMK2G*) and vinculin (*VCL*) are located outside the 5′ and 3′ ends of the duplicated region.

PATHOGENESIS OF QPD

The hallmark feature of QPD, implicated in its pathogenesis, is the more than 100-fold increases in uPA mRNA and protein levels in platelets,[2–4] despite normal or minimal changes in plasma uPA.[4,5] However, it is not yet known why the one extra copy of the gene in persons with QPD leads to such tremendous increases in platelet uPA.[7] Indeed, an extra copy of the gene would only be expected to increase uPA mRNA levels by ~50%,[7] which is consistent with the levels of uPA mRNA seen in QPD CD34 + cells and saliva.[2,3]

During normal megakaryocyte differentiation, uPA expression persists at fairly low levels whereas the overexpression of uPA emerges as QPD hematopoietic progenitors differentiate into megakaryocytes and platelets.[2] The increases in uPA mRNA come from the disease chromosome,[3] consistent with a cis-regulatory defect of either one or both copies of *PLAU* on the disease chromosome.[7]

The overexpression of uPA in megakaryocytes leads to uPA storage in α-granules.[2] uPA is a fairly selective serine protease that activates plasminogen to plasmin and is normally inhibited by plasminogen activator inhibitor-1 (PAI-1).[1] Platelets contain large amounts of active PAI-1 but these stores are fully consumed by complex formation with active uPA in QPD platelets, and the amounts of uPA in QPD platelets require additional PAI-1 for full neutralization.[4] In QPD platelets, most of the uPA is present in active, two chain and low-molecular weight uPA, which are generated by proteolysis of single chain uPA.[1,2,4] QPD platelets but not plasma contains increased levels of plasmin-α2 plasmin inhibitor complexes, consistent with intraplatelet but not systemic plasmin generation.[17] Intraplatelet plasmin generation is thought to be the reason why most of the proteins stored in QPD α-granules are abnormally proteolyzed, including factor V, multimerin 1, thrombospondin 1, von Willebrand factor, fibrinogen, fibronectin, osteonectin, and P-selectin.[2,4,9–12,17] The plasmin-mediated degradation of

QPD α-granule proteins may occur late, perhaps even in circulating platelets, as QPD megakaryocytes grown in culture (which do not contain plasmin or plasminogen) synthesize and store undegraded α-granule proteins.[2]

MECHANISM OF QPD BLEEDING

Individuals with QPD have several defects that could theoretically contribute to bleeding including increased platelet uPA, the loss (due to proteolysis) of α-granule proteins (including factor V), a mild reduction in platelet numbers (which has an unknown cause), and defective platelet-aggregation function.[4,9,11–13] However, dramatic responses to fibrinolytic inhibitors[13] in QPD individuals suggest that accelerated fibrinolysis is a key mediator of QPD bleeding.

Studies of coagulation and fibrinolytic proteins in persons with QPD have identified few abnormalities apart from an increased platelet content of uPA,[8–11] which contrasts with the normal amounts of uPA in QPD plasma (prepared with platelet-activation inhibitors)[4,5] and urine.[6] The levels of plasminogen and tissue plasminogen activator are normal in QPD blood.[2,17] The containment of uPA in QPD platelets is probably very important for preventing systemic fibrinolysis in QPD as mice that overexpress uPA in megakaryocytes have a QPD-like gain-of-function defect in fibrinolysis, without the systemic fibrinolysis that occurs in mice that overexpress uPA in the liver.[18,19] In vitro, the increased uPA released by QPD platelets triggers accelerated lysis of forming or preformed fibrin clots.[5] QPD platelet-rich fibrin clots have an initial normal gross and microscopic appearance; however, the increased uPA released by QPD platelets leads to accelerated loss of clot integrity, with dissolution of fibrin and platelet-fibrin contacts, and marked increases in D-dimer generation.[5]

Table 1 Laboratory Findings in QPD

Laboratory Test	Finding
Platelet count	Reduced or normal (80–245 × 10^9/L)
Other blood counts	Normal unless iron deficient
PFA-100® closure times	Normal
Bleeding time	Normal to mildly prolonged
Coagulation and fibrinolysis parameters	
PT (INR)	Normal
aPTT	Normal
Fibrinogen	Normal
D-dimer	Normal
uPA	Normal in plasma prepared with platelet activation inhibitors
	Elevated in platelets (~400–600 ng uPA/10^9 platelets)
uPA-plasminogen activator inhibitor 1 complexes	Normal in plasma
	Elevated in platelets
Plasmin-α2 plasmin inhibitor complexes	Normal in plasma
	Elevated in platelets
Thromboelastography (whole blood or platelet rich plasma)	Normal
Light transmission platelet aggregometry	Nondiagnostic findings
Epinephrine	Primary response absent, reduced or normal, with absent secondary aggregation
ADP	Normal to reduced
Collagen	Normal to reduced
Arachidonic acid	Normal
Thromboxane analogue U46619	Normal to reduced
Ristocetin	Normal
Platelet glycoprotein analysis	
Western blot analysis for α-granule protein degradation and platelet uPA	α-Granule protein degradation and increased platelet uPA
Plasma thrombopoietin levels	Normal (7.65 ± 2.9 pg/mL; reference interval 2.78–18.5 pg/mL)
Genetic tests for the QPD *PLAU* duplication mutation	Positive (duplicated and normal alleles detected)

QPD, Quebec platelet disorder; PFA, Platelet Function Analyzer; PT (INR), prothrombin time (international normalized ratio); aPTT, activated partial thromboplastin time; uPA, urokinase plasminogen activator ADP, adenosine diphosphate.

APPROACH TO THE DIAGNOSIS OF QPD

QPD should be suspected when there is a personal and/or family history of an autosomal dominant bleeding disorder with features typical of the disorder (as outlined in the section on clinical features) including a person or family history of challenge-related bleeding that is delayed in onset and only responds to fibrinolytic inhibitor therapy; large, trauma-induced bruises or hematomas; joint bleeds; and spontaneous hematuria.[1,13] Some persons with QPD without significant hemostatic challenges, or who received fibrinolytic inhibitor treatment for all challenges, may have no personal history of bleeding, apart from their positive family history.[13]

The findings for most hemostasis investigations are nondiagnostic in QPD (Table 1). For example, bleeding times and closure times measured by the Platelet Function Analyzer 100® (Siemens Healthcare Diagnostics, Deerfielf, IL) can be normal.[1,9,10] Platelet counts are typically half of the value of unaffected individuals and thus range from mildly reduced to normal.[13] The prothrombin time (or international normalized ratio [INR]) and activated partial thromboplastin time are normal if the sample is processed to avoid loss of factor V activity from uPA-induced plasmin generation ex vivo.[1,8–10] Plasma tests for systemic fibrinolysis (e.g., fibrinogen, D-dimer, plasminogen, uPA, uPA-plasminogen activator inhibitor 1 complexes, and plasmin-α2 plasmin inhibitor complexes) are normal in QPD.[4,5,12,17] Whole blood and platelet-rich plasma thromboelastography findings are normal as the test is not sensitive to the amounts of uPA in QPD blood.[5]

Platelet-aggregation findings in QPD show nonspecific abnormalities, including absent primary or secondary aggregation with epinephrine, with or without reduced aggregation with adenosine diphosphate and collagen.[1,9,10] Plasma thrombopoietin levels are normal in QPD (Table 1).

Genetic testing for the *PLAU* duplication mutation[7] has become the method of choice for definitive diagnosis of QPD. The QPD duplication mutation can be detected by several approaches, including polymerase chain reaction (PCR) assays for the breakpoint sequence (evaluated by gel fragment or real-time endpoints) and Southern blotting.[7] The PCR assay for the QPD mutation can be performed on cord blood samples to rapidly determine if a newborn of an affected parent has QPD.[7] Assays for increased platelet uPA and for platelet α-granule protein degradation,[4,9,10,12,13,17,20] which are not widely available, have been used to diagnose QPD and they would be appropriate assays to evaluate a QPD-like bleeding syndrome if the described mutation was not present.

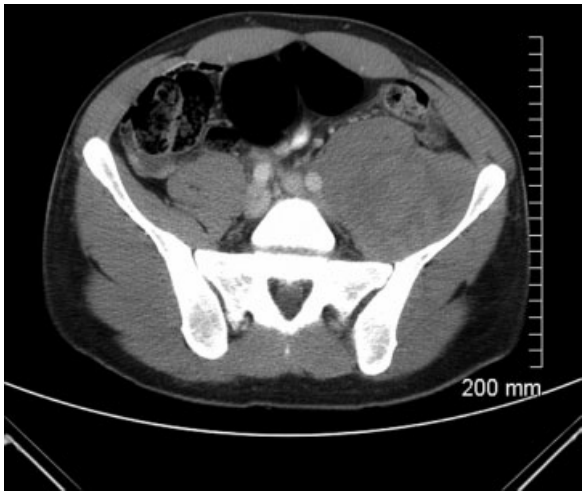

Figure 3 Abdominal computerized axial tomography scan of a severe bleeding episode in a person with QPD. The image shows a large bleed on the right side involving the psoas muscle.

MANAGEMENT OF QPD BLEEDING

Currently, there is only one known treatment for QPD: treatment with an antifibrinolytic drug.[13] The most commonly used therapy is tranexamic acid at the typical doses used for bleeding disorder management (25 mg/kg body weight, every 8 hours for persons with normal renal function; the dose can be reduced to 15 to 20 mg/kg body weight every 8 hours if the full dose is not

Table 2 Recommended Treatments for Prevention and Control of QPD Bleeding

Indication	Length of Treatment with Tranexamic Acid or Aminocaproic Acid
Minor bleeding	3–4 d
Bleeding after major trauma	5–7 d
Menorrhagia	Treatment during menses
Intracranial bleeding	10–14 d
Joint bleeds	5–7 d; for prophylaxis, several weeks to several months, as needed
Spontaneous hematuria	No treatment
Pregnancy and uncomplicated vaginal childbirth	No treatment
Dental extractions	4–5 d
Minor surgery (e.g., biopsies)	3–4 d
Major surgery	5–7 d including an intravenous dose before surgery

QPD, Quebec platelet disorder; d, day.

tolerated). This drug has been used successfully for managing QPD bleeding, including severe bleeding episodes such as the psoas bleed shown in Fig. 3. Aminocaproic acid can also be used but this requires higher doses because it is less potent than tranexamic acid at inhibiting fibrinolysis. Some patients have recurrent joint bleedings that may benefit from prophylactic treatment with antifibrinolytic agents at standard doses but at reduced dosage frequency of once to twice a day. For surgeries with high risks of thrombosis (e.g., hip or knee replacement), we recommend continuing with the antifibrinolytic therapy until anticoagulant prophylaxis (given at standard doses) is discontinued. Table 2 summarizes the duration of treatment for preventing and treating QPD bleeding.

CONCLUSION

QPD is the only platelet disorder attributed to a gain-of-function defect in fibrinolysis,[5] resulting from increased expression and storage of uPA in megakaryocytes.[2–4] QPD is also the only known bleeding disorder known to result from a gain-of-copy number mutation.[7] The reason for the reduction in platelet counts in the disorder is not yet known. The knowledge that QPD results from a tandem duplication of *PLAU* has advanced the application of molecular diagnostics to this condition, which at the moment, has only one known genetic cause.[7] However, important issues need to be resolved to better understand QPD pathogenesis, including the mechanisms by which one extra copy of *PLAU* leads to tremendously increased uPA expression during megakaryopoiesis but not in hematopoietic progenitors, plasma or urine.[2,3,7] Clinicians need to consider QPD as a potential diagnosis for individuals with low or normal platelet counts who have an unexplained, personal, and/or family history of delayed bleeding. More information is needed on the prevalence of QPD as a cause of bleeding in North American and in other regions of the world, where unexplained bleeding problems are quite common.

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