Genetic Analysis and Functional Characterization of Prothrombins Corpus Christi (Arg382-Cys), Dhahran (Arg271-His), and Hypoprothrombinemia

By Aengus S. O’Marcaigh, William L. Nichols, Nancy L. Hassinger, James D. Mullins, Ahmad A. Mallouh, Gerald S. Gilchrist, and Whyte G. Owen

The structural abnormalities and functional characteristics of dysfunctional prothrombin variants in two new kindreds have been determined. Prothrombin Corpus Christi (family 1) was purified and found to have markedly reduced fibrinogen clotting activity, yet normal amidolytic and near-normal platelet aggregating activity. A transition (C to T) at nucleotide position 8986, present in the heterozygous form in affected family members, resulted in the substitution of Cys for Arg at codon 271. This substitution results in the loss of a positive charge within the fibrinogen-binding exosite of thrombin, thus accounting for the observed functional defect. A heterozygous C to T transition was also present at position 19984 in other family members with a hypoprothrombinemic phenotype. This mutation results in the replacement of Gln at codon 8885 (CAA) by a premature stop codon (TAA). Prothrombin Dhahran (family 2) was found to have markedly reduced fibrinogen clotting activity, but normal amidolytic activity. Affected family members were found to have a G to A transition at nucleotide position 7312 resulting in the substitution of His for Arg 271. This substitution results in the abolition of a factor Xa cleavage site, yielding meizothrombin rather than thrombin, on activation of prothrombin Dhahran by factor Xa. All but one of the above mutations occur at CpG dinucleotides, thus further supporting the observation of a high incidence of CpG transitions in hereditary dysprothrombinemia. The significant bleeding tendencies of individuals homozygous for prothrombin Dhahran (prothrombin clotting activity 5% to 7%) contrast sharply with the absence of significant chronic bleeding in the proband expressing prothrombin Corpus Christi (prothrombin clotting activity 2%). Our findings underscore the capacity of thrombin to contribute to clinical hemostasis by mechanisms other than its fibrinogen clotting activity.

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HUMAN PROTHROMBIN is a 579-amino-acid single-chain vitamin K-dependent plasma glycoprotein (MW 71,600). Proteolytic cleavage of prothrombin by thrombinase yields the active serine protease thrombin, which consists of two polypeptide chains, covalently linked by a disulfide bridge. Thrombin catalyzes the processes of clotting and platelet activation and further regulates hemostasis by activating coagulation proteins such as factors V, VIII, IX, and protein C.

Hereditary prothrombin deficiency is a rare disorder expressed either as hypoprothrombinemia (a deficiency of a functionally normal protein) or as dysprothrombinemia (the production of a dysfunctional prothrombin variant). Fewer than 20 families with hereditary hypoprothrombinemia have been described and the responsible mutation has been reported in only three. Twenty-five hereditary dysfunctional prothrombin variants have been reported. Although the structural abnormality is known for eight of these variants (Fig 1), the responsible mutation has been identified for only five. The identification of additional mutations is important not only for genetic counseling, but also because the characterization of the functional impairment resulting from a specific structural defect can provide valuable insights into the structure-function relationship of thrombin.

We have identified and characterized two new dysfunctional prothrombin variants, prothrombin Corpus Christi (family 1) and prothrombin Dhahran (family 2). This report describes the mutations responsible for these variants and for hereditary hypoprothrombinemia, defines the variant proteins’ structural and functional defects, and correlates this information with the observed clinical phenotype.

CLINICAL REPORTS

Family 1. A 21-year-old Hispanic man with no history of abnormal bleeding was evaluated for prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT). The patient did not report any bleeding or bruising tendency and had previously undergone skin grafting, extraction of several molar teeth, and had a deep laceration sutured without excessive bleeding. Figure 2 shows the pedigree of family 1 and Table 1 outlines the results of coagulation testing. Assays of all other coagulation factors of the proband were normal. The proband (II.14) and two deceased siblings (II.3, II.6) experienced a similar hemorrhagic vesicular rash of the lower extremities in early childhood. The proband required prolonged hospitalization and skin grafting of the skin lesions during infancy. These lesions resolved completely during early childhood leaving atrophic scars on both thighs. The proband has recently been experiencing the development of intermittent painful raised erythematous lesions on the lower extremities. These lesions have not resulted in skin breakdown or scarring and have resolved promptly after the administration of fresh frozen plasma. Two siblings (II.3, II.6) died at 24 years of age following hemorrhagic events (II.3 of cerebral hemorrhage and edema after head trauma, and II.6 of cerebral hemorrhage 2 weeks after the birth of her third child). Both had markedly prolonged PT (51s and 43s, respectively) and APTT (65s and 45s, respectively) before these events. The proband’s father (I.3) had no history of abnormal bleeding and died of prostate cancer at 45 years of age. The proband’s mother (I.4) had no history of abnormal bleeding. None of the other immediate and antecedent family members had a history of abnormal bleeding.

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Submitted March 4, 1996; accepted May 29, 1996.

Supported by Grant No. HL47469 from the National Heart, Lung, and Blood Institute and by Mayo Foundation for Medical Education and Research.

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Blood, Vol 88, No 7 (October 1), 1996: pp 2611-2618
Family 2. A lifelong moderate bleeding tendency characterized by occasional spontaneous hemorrhages, muscle bleeds and postsurgical hemorrhage was present in four members (III.1, III.2, III.6, and III.7) of a Saudi Arabian family (Fig 3). These individuals had prolongations of PT and APTT, which are shown along with prothrombin antigen and activity levels in Table 1. Assays of all other coagulation factors of the proband (III.7) were normal. All other family members were asymptomatic. Double consanguinity was present between both parents.

MATERIALS AND METHODS

Clotting times and factor assays. Venous blood was anticoagulated with sodium citrate (0.38% final concentration). Platelet-poor plasma was prepared by centrifugation at 1,700g at 4°C for 10 minutes and was stored at −40°C to −70°C. Coagulation assays were performed using an ACL 300 coagulometer (Instrumentation Laboratory, Lexington, MA) for assays of coagulation factors I, II, V, VII and X, and a Coa Screener instrument (American Labor, Raleigh, NC) for PT, APTT, and coagulation factors VIII, IX, XI and XII. The reagents used were: Thromboplastin C (ISI 2.9, Baxter-Dade, Deerfield, IL) for PT for family 1; Innovin thromboplastin (ISI 1.0, Baxter-Dade) for PT for family 2; PT/fibrinogen reagent (Instrumentation Laboratory) for factors I, II, V, VII, and X; Automated aPTT reagent (Organon Teknika, Durham, NC) for APTT and factors VIII, IX, XI and XII. Prothrombin-deficient plasma (Baxter-Dade) was used to assay prothrombin activity. Prothrombin antigen was measured by the quantitative immunoelectrophoretic method of Laurell, using antihuman prothrombin serum (Behring Diagnostics, Somerville, NJ) or IgG (The Binding Site, Birmingham, UK).

Prothrombin purification, activation, and characterization. Plasma was obtained from the proband in family 1 by plasmapheresis after obtaining the written informed consent of the proband and the approval of the Mayo Clinic Institutional Review Board. Prothrombin was purified from human plasma by a modification of the method of Owen with separation of the vitamin K–dependent proteins by isocratic elution (0.3 mol/L NaCl) from a QAE Sephadex column. Purified proteins were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Phastgel system (Pharmacia, Alameda, CA).

Prothrombin was activated at room temperature in a reaction mixture containing 0.3 mg/mL purified human prothrombin, 20 μg/mL taipan snake venom (Sigma, St Louis, MO), 40 μg/mL phosphatidyl-
Table 1. Coagulation Studies on Members of the Families With Prothrombin Corpus Christi and Hypoprothrombinemia (family 1) and Prothrombin Dhahran (family 2)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/Sex</th>
<th>PT (10 to 12 s)</th>
<th>APTT (25 to 35 s)</th>
<th>Prothrombin Antigen Activity (72% to 140%)</th>
<th>Prothrombin Activity (70% to 140%)</th>
<th>Phenotype</th>
</tr>
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<tbody>
<tr>
<td>I.4</td>
<td>58/F</td>
<td>11.2</td>
<td>—</td>
<td>50</td>
<td>65</td>
<td>Heterozygote (hypo)</td>
</tr>
<tr>
<td>II.1</td>
<td>38/F</td>
<td>11.2</td>
<td>—</td>
<td>42</td>
<td>54</td>
<td>Heterozygote (hypo)</td>
</tr>
<tr>
<td>II.4</td>
<td>30/M</td>
<td>11.0</td>
<td>—</td>
<td>98</td>
<td>131</td>
<td>Normal</td>
</tr>
<tr>
<td>II.5</td>
<td>25/M</td>
<td>12.0</td>
<td>—</td>
<td>93</td>
<td>110</td>
<td>Normal</td>
</tr>
<tr>
<td>II.9</td>
<td>23/M</td>
<td>11.0</td>
<td>—</td>
<td>85</td>
<td>69</td>
<td>Heterozygote (dys)</td>
</tr>
<tr>
<td>II.10</td>
<td>22/M</td>
<td>11.2</td>
<td>—</td>
<td>44</td>
<td>54</td>
<td>Heterozygote (hypo)</td>
</tr>
<tr>
<td>II.14</td>
<td>21/M</td>
<td>68.0</td>
<td>110</td>
<td>25</td>
<td>2</td>
<td>Compound heterozygote (hypo &amp; dys)</td>
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</table>

Family 2—Prothrombin Dhahran

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/Sex</th>
<th>PT (10 to 12 s)</th>
<th>APTT (25 to 35 s)</th>
<th>Prothrombin Antigen Activity (72% to 140%)</th>
<th>Prothrombin Activity (70% to 140%)</th>
<th>Phenotype</th>
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<td>11.7</td>
<td>36</td>
<td>114</td>
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<tr>
<td>II.2</td>
<td>32/F</td>
<td>11.4</td>
<td>37</td>
<td>107</td>
<td>70</td>
<td>Heterozygote (dys)</td>
</tr>
<tr>
<td>II.3</td>
<td>22/F</td>
<td>10.1</td>
<td>26</td>
<td>116</td>
<td>108</td>
<td>Normal</td>
</tr>
<tr>
<td>III.1</td>
<td>14/F</td>
<td>17.1</td>
<td>73</td>
<td>110</td>
<td>7</td>
<td>Homozygote (dys)</td>
</tr>
<tr>
<td>III.2</td>
<td>11/F</td>
<td>17.3</td>
<td>97</td>
<td>84</td>
<td>7</td>
<td>Homozygote (dys)</td>
</tr>
<tr>
<td>III.3</td>
<td>9/F</td>
<td>10.8</td>
<td>27</td>
<td>84</td>
<td>100</td>
<td>Normal</td>
</tr>
<tr>
<td>III.4</td>
<td>8/F</td>
<td>10.5</td>
<td>38</td>
<td>109</td>
<td>100</td>
<td>Normal</td>
</tr>
<tr>
<td>III.5</td>
<td>6/M</td>
<td>10.7</td>
<td>40</td>
<td>94</td>
<td>55</td>
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<td>III.6</td>
<td>4/F</td>
<td>18.3</td>
<td>82</td>
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<tr>
<td>III.7</td>
<td>3/F</td>
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<td>93</td>
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<td>7/M</td>
<td>11.5</td>
<td>41</td>
<td>106</td>
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<td>Heterozygote (dys)</td>
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<tr>
<td>III.9</td>
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<td>10.6</td>
<td>31</td>
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<td>III.10</td>
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<td>10.5</td>
<td>30</td>
<td>106</td>
<td>97</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Fibrinogen clotting activity: 1.7%
Amidolytic activity: 97%
Prothrombin Corpus Christi: 5% to 7%
Prothrombin Dhahran: 98%

Abbreviations: hypo, hypoprothrombinemia; dys, dysprothrombinemia.

Fig 3. Pedigree of family 2 (Prothrombin Dhahran) showing prothrombin antigen and activity levels of family members including the proband (III.7, arrow). *Blood samples were not available from these family members.
choline and phosphatidyl-serine (5:1) vesicles, 4 mmol/L CaCl$_2$, 0.2 mol/L NaCl, and 20 mmol/L Tris-HCl pH 7.8. Thrombin was isolated from the activation mixture by gradient elution from sulfopropyl Sephadex after a 2-hour incubation.

Thrombin amidolytic activity was determined by measuring the rate of change in absorbance at 405 nm after the addition of a synthetic substrate (25 μL of 1 mmol/L Spectrozyme TH, American Diagnostica, Greenwich, CT) to 1 mL thrombin solutions in 100 mmol/L NaCl, 100 mmol/L Tris-HCl, 0.1% PEG pH 8.0. The amidolytic activity of activated prothrombin Dhabran was determined by the addition of 2 μL taipan snake venom (1mg/mL in 0.15 mol/L NaCl) and 4 μL CaCl$_2$ (100 mmol/L) to 100 μL of plasma diluted with an equal volume of water. Aliquots of 10 μL were removed at intervals and diluted in 185 μL of 100 mmol/L NaCl, 100 mmol/L Tris-HCl, 0.1% PEG pH 8.0 to stop the reaction. This solution was added 5 μL of Spectrozyme TH, and the rate of generation of amidolytic activity was determined by spectrophotometry as described above.

Fibrinogen clotting activity of purified human thrombin was determined by measurement of fibrinopeptide A and B release. Thrombin was incubated at 23°C in a solution containing 2 μmol/L human fibrinogen, 100 mmol/L NaCl, 100 mmol/L Tris-HCl, 0.1% PEG pH 8.0. The reaction was stopped at intervals by the addition of 7.5% HClO$_4$ to bring the final concentration to 5%. Samples were microcentrifuged for 3 minutes, and fibrinopeptide A and B concentrations were determined by high performance liquid chromatography.

Platelet aggregation. Platelets were isolated from human blood by a modification of the method of Mustard et al. and resuspended in Tyrode’s solution buffered with 20 mmol/L NaHEPES [4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid]. Aggregation studies were performed using a dual channel Payton aggregation module (Payton Assoc, Buffalo, NY).

Genetic analysis. Genomic DNA was purified from peripheral blood leukocytes in frozen blood by the method of Gustafson et al. Segments of the human prothrombin gene were amplified by polymerase chain reaction (PCR) using an automated thermal cycler (Perkin Elmer, Norwalk, CT). The amplification primers used are illustrated in Table 2. A T7 phage promoter sequence (5’-TAATACGACTCAGATATTAG-3’) was incorporated into the 5’ end of the upstream (sense) primer of each amplification primer set. An SP6 phage promoter sequence (5’-AATATACGACTATATAGG-3’) was incorporated into the 5’ end of the downstream (antisense) amplification primer. The reaction mixture contained 250 ng genomic DNA and 0.5 U T7A DNA polymerase in a 25-μL reaction volume of 1.0 to 3.0 mmol/L MgCl$_2$, 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 8.3, 200 μmol/L each dNTP, and 0.5 μmol/L each amplification primer. Thirty PCR cycles were performed consisting of denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes, and elongation at 72°C for 3 minutes.

Amplified products were transcribed to single stranded RNA by the method of Stofflet et al using T7 or SP6 RNA polymerase. Transcripts of all 14 exons of the prothrombin gene from members of both families were then screened for the presence of mutations by dideoxy fingerprinting (ddf). Ddf is a sensitive mutation detection technique which involves a Sanger dideoxy sequencing reaction using one dideoxy nucleotide and an end-labeled sequencing primer followed by non-denaturing gel electrophoresis. Because of the generation of a ladder of bands, this technique is more sensitive than single-stranded conformational polymorphism analysis (SSCP), and it has been shown to have detected 84 of 84 different mutations of the human factor IX gene with a very low rate of false-positive signals. Abnormal transcripts were sequenced by genomic amplification with transcript sequencing (GAWTS) using nested ddf end-labeled sequencing primers (Table 2). All detected mutations were confirmed by sequencing of the antisense transcript. The nucleotide numbering sequence is that of Degen et al. 19

RESULTS

Family 1: Prothrombin Corpus Christi. The proband had marked prolongation of the PT and APTT (Table 1). The PT of other family members was normal. Prothrombin antigen levels and functional activity levels for family members are listed in Table 1 and depicted in Fig 2. The proband (II.14) had a prothrombin antigen level of 25% with a disproportionately lower functional activity of 2%, consistent with hypoprothrombinemia and dysprothrombinemia. Three family members (I.4, II.1, and II.10) exhibited a moderate and proportionate reduction in both antigen and activity levels, consistent with hypoprothrombinemia. One sibling (II.9) had a low-normal antigen level (85%) with disproportionately lower functional activity level of 69%, consistent with dysprothrombinemia. Two other siblings (II.4, II.5) had normal antigen and activity levels.

Purification and characterization. The migration of the proband’s purified prothrombin on SDS-PAGE was identical to that of normal human prothrombin. Activation of purified prothrombin with taipan snake venom proceeded at a normal rate and yielded activation products of identical molecular weight to those of normal human prothrombin. The specific fibrinogen clotting activity of the purified thrombin as determined by measurement of fibrinopeptide release was 1.7% that of normal human thrombin. Its specific activity for hydrolysis of chromogenic polypeptide substrates was 97% that of normal human thrombin. The purified thrombin exhibited a mild reduction in its ability to activate human platelets as determined by comparison of platelet aggregation and adenosine triphosphate (ATP) secretion responses. After stimulation with thrombin Corpus Christi, platelet ATP secretion was 35% of that released after stimulation with an identical concentration of normal human thrombin (data not shown).

Genetic analysis. Dideoxy fingerprinting of exon 10 showed three distinct migration patterns among family members. Transcripts sequencing of this exon showed two point mutations. The first mutation (Fig 4), a C to T transition at nucleotide position 8885 (C8885T), was present in heterozygous form in the proband (II.14) and in one sibling (II.9). The second mutation, a G to A transition at nucleotide position 8845 (G8845A), was present in the heterozygous form in one family member (I.4), and in all three siblings (II.4, II.5, and II.9) who inherited the normal (nonhypoprothrombinemic) maternal allele. Dideoxy fingerprinting of exon 14 showed an abnormal migration pattern for the mother (I.4), the proband (II.14), and two siblings (II.1,II.10). Transcripts sequencing of this exon showed a C to T transition at nucleotide position 19994 (C19994T) in the heterozygous form in each of these family members (I.4, II.1, II.10, and II.14) (Fig 5). This mutation was absent in all other family members.

Family 2: Prothrombin Dhabran. Prolongation of both PT and APTT was present in four family members (III.1, III.2, III.6, and III.7), whereas others (II.1, II.2, III.5, and
Table 2. Oligonucleotide Primer Sequences Used to Amplify, Screen, and Sequence Exons of the Prothrombin Gene

<table>
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<tr>
<th>Exon(s)</th>
<th>Primer</th>
<th>Primer Position*</th>
<th>Oligonucleotide Primer Sequence</th>
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<tbody>
<tr>
<td>1 &amp; 2</td>
<td>Amp</td>
<td>274-1072</td>
<td>5'-GGACAGGAGGACTATC-3'</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5'-GGAAGTGTCTAGAGTT-3'</td>
</tr>
<tr>
<td>3 &amp; 4</td>
<td>Amp</td>
<td>1626-2026</td>
<td>5'-GGGAGGAGGATACATGAGTA-3'</td>
</tr>
<tr>
<td></td>
<td>Seq</td>
<td>1659-1717</td>
<td>5'-ACAGGAGGCGGTGTA-3'</td>
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<tr>
<td>5 &amp; 6</td>
<td>Amp</td>
<td>1539-2019</td>
<td>5'-ACTCCAGCTCATCCT-3'</td>
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<td></td>
<td>Seq</td>
<td>1909-2011</td>
<td>5'-CCAAACCCGTGTCAG-3'</td>
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<td>13 &amp; 14</td>
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<td>5'-CATGCACGCTAACT-3'</td>
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<td>14</td>
<td>Seq</td>
<td>20407-20422</td>
<td>5'-ATGCTGTCTTGCAGTG-3'</td>
</tr>
</tbody>
</table>

Abbreviations: Amp, amplification primer; Seq, sequencing primer.
* Nucleotide position given for each primer is the position of the genomic nucleotide immediately 3' of the primer 3' end; numbering system is that of Degen et al.10.
† T7 and SP6 phage promoter sequences incorporated into amplification primers at these sites.

III.8) exhibited a slightly prolonged APTT only (Table 1, Fig 3). All family members had a normal level of prothrombin antigen, but prothrombin functional activity was either markedly decreased (5% to 7%), moderately decreased (52% to 70%), or normal (Fig 3). This pattern of findings suggests that family members III.1, III.2, III.6, and III.7 are homozygous and that II.1, II.2, III.5, and III.8 are heterozygous for a dysprothrombinemic mutation. The maximal amidolytic activity of plasma from the proband (III.7), and the rate of generation of this activity on activation with taipan snake venom, were identical to that of pooled normal human plasma.

Genetic analysis. Sequencing of exon 8 of the prothrombin gene of the proband (Fig 6) showed a G to A point mutation at nucleotide position 7312 (G7312A). This was present in the homozygous form in all four severely affected family members (III.1, III.2, III.6 and III.7), in the heterozygous form in mildly affected family members (II.1, II.2, III.5 and III.8), and was absent in all other family members. These findings confirm the autosomal recessive inheritance pattern suggested by the above clotting studies.

DISCUSSION
The newly identified prothrombin variants have been designated prothrombin Corpus Christi (family 1) and prothrombin Dhahran (family 2) in accordance with the convention

![Fig 4. Heterozygous C to T transition at nucleotide position 8885 (C8885T) within exon 10 which is responsible for prothrombin Corpus Christi (family 1).](image-url)
of naming such variants after the proband’s city of origin. The normal activation kinetics of purified prothrombin Corpus Christi and the generation of activation products of normal molecular weight indicated that the functional defect of this variant resides in the thrombin portion of the molecule. The preservation of normal amidolytic activity despite a markedly reduced fibrinogen clotting activity further suggested a functional defect within the fibrinogen binding exosite. The identification of a C to T transition at nucleotide position 8885 in the proband and one sibling confirms the presence of a structural abnormality at this site. This mutation results in the replacement of Arg382(CGC) by Cys(TGC) and the loss of a critical positive charge within the fibrinogen binding exosite thus explaining the functional characteristics of prothrombin Corpus Christi.

The substitution of Cys for Arg382 has been shown by protein sequencing also to be responsible for thrombin Quick I.20 The functional characteristics of thrombin Quick I are very similar to those of prothrombin Corpus Christi and it is likely that the same point mutation is responsible for both variants. All siblings believed to express prothrombin Corpus Christi alone experienced skin lesions of the lower extremities similar to those experienced by the proband with thrombin Quick I (personal communication, Dr Donald E. Macfarlane, March 1995). The pathophysiologic basis for these dermatologic lesions is not clear and warrants further study.

The moderate-to-severe bleeding tendency of the proband with prothrombin Dhahran (prothrombin clotting activity 5%) contrasts sharply with the absence of a significant bleeding tendency in the proband with prothrombin Corpus Christi who exhibited a lower prothrombin clotting activity of 2%. This failure of the one-stage assay of prothrombin activity to predict the degree of clinical bleeding in patients with dysfunctional prothrombin variants has also been observed in persons with prothrombin Gainesville who exhibit a significant bleeding tendency despite prothrombin activities of 23% and 25%.22 Because the one-stage assay is a reflection only of the direct fibrinogen clotting activity of prothrombin, it is possible that a relative preservation of activity toward other known or unknown substrates might explain this disparity and, therefore, the absence of a major bleeding tendency in the proband with prothrombin Corpus Christi. A more reliable correlation between prothrombin activity and clinical bleeding tendency exists in patients with hypoprothrombinemia where the reduction in fibrinogen clotting activity is paralleled by an equivalent reduction in activity toward all other substrates. The preservation of prothrombin Corpus Christi’s capacity to activate platelets relative to its markedly reduced fibrinogen clotting activity undoubtedly contributes to the absence of a significant bleeding tendency. The relative contributions of the many roles of thrombin in the maintenance of hemostasis have not been firmly established. Our findings indicate that thrombin can contribute significantly to clinical hemostasis by mechanisms other than its direct fibrinogen clotting activity.

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273 Ala
272 Thr
271 Arg
270 Gly
Brother (III.4) Normal
Proband (III.7) Homozygote

---

Fig 5. Heterozygous C to T transition at nucleotide position 19994 (C19994T) within exon 14 which is responsible for hypoprothrombinemia (family 1).

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Fig 6. Homozygous G to A point mutation at nucleotide position 7312 (G7312A) within exon 8 which is responsible for prothrombin Dhahran (family 2).
The identification of a C to T transition at nucleotide position 19994 that generates a stop codon is the first time a mutation within the thrombin coding sequence has been shown to be responsible for hypoprothrombinemia. All three previously reported mutations causing hypoprothrombinemia occurred close to the 5' terminal resulting in a severely truncated protein without a thrombin domain. The stop codon we describe occurs at the site of a highly conserved residue Gin 541. Although this residue is very close to the carboxyl terminal of the mature protein, the truncated protein could not be identified immunologically or purified from the patient’s plasma. It is likely that this mutation interferes with transcript stability, translation or posttranslational processing and secretion, or that the truncated protein is rapidly cleared from the circulation.

The mutation responsible for prothrombin Dhahran is a G to A transition at nucleotide position 7312. This results in the replacement of Arg 271 (CGT) by His (CAT). Arg 271 is a highly conserved residue at one of the sites at which prothrombin is cleaved by factor Xa (Arg 271-Thr). This substitution prevents cleavage by factor Xa at this site and results in the generation of meizothrombin, a normally transient intermediate of prothrombin activation. A stable form of meizothrombin generated by activation of a recombinant prothrombin (Arg 271-Ala) has been shown to have 7% the fibrinogen clotting activity of normal human alpha thrombin. This activity is identical to that observed in the plasma of the family members homozygous for prothrombin Dhahran after activation with Taipan snake venom. A similar mutation has been demonstrated in the heterozygous form in the proband with prothrombin Padua. The functional defect of prothrombin Dhahran is similar to that of prothrombins Barcelona/Madrid (Arg 271-Cys). A previously unreported polymorphism consisting of a G to A transition was detected at nucleotide position 8845 within exon 10 in the normal (nonhypoprothrombinemic) maternal allele of family 1. This occurs in the codon coding for Pro 368 (CCG to CCA) and does not result in a change in amino acid sequence. This polymorphism does result in the disruption of an AcI I endonuclease digestion site (5' CCGC 3'). Three of the four point mutations reported here occur at CpG dinucleotides in the coding strand (C8885T Prothrombin Corpus Christi) or in the noncoding strand (G7312A Prothrombin Dhahran, and the G8845A polymorphism). Therefore, CpG dinucleotide transitions account for the genetic defect in six of eight kindreds with dysprothrombinemia in whom the genetic defect has been demonstrated. This type of transition occurs with increased frequency at selected CpG sites in the factor IX gene, and is also responsible for some dysfunctional antithrombin III and fibrinogen variants. The techniques of GAWTS and ddF used in this report have previously been used to define the molecular genetic defects in a large number of patients with hemophilia B. This report serves to illustrate their use in the genetic analysis of other hereditary coagulation factor deficiencies and also contributes to our growing understanding of the spectrum of mutations responsible for congenital hypoprothrombinemia and dysprothrombinemia.

Our data confirm current understanding of some aspects of the structure-function relationship of thrombin and underscore the capacity of thrombin to contribute significantly to hemostasis independently of its fibrinogen clotting activity. The association of the Corpus Christi mutation with an inflammatory skin process suggests further that thrombin has some role in inflammation or vascular physiology unrelated or peripherally related to hemostasis.

ACKNOWLEDGMENT

Pamela K. Fisher, Todd M. Daniels, and our other coagulation laboratory technologists provided expert diagnostic assistance. Pamela A. Krain provided expert secretarial support.

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Genetic analysis and functional characterization of prothrombins Corpus Christi (Arg382-Cys), Dhahran (Arg271-His), and hypoprothrombinemia

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