Supplementary Fig. S1 Pedigrees of the 48 PS-deficient families in which the causative PROS1 defect has been identified. The arrow denotes the probands. Round symbols, females; square symbols, males; half-filled symbols, subjects with heterozygous PROS1 mutation; full-filled symbols, subjects with compound heterozygous PROS1 mutations; symbols with diagonal, deceased family members; #, subjects with a history of venous thromboembolism; ▲, subjects with skin bruise discoloration, ulcer, varicose veins or skin necrosis; ▼, subjects with myocardial infarction or cerebral infarction; PS: A, protein S activity; FPS: Ag, free protein S antigen; ND, no detection for PROS1 mutation; †, novel mutation. Reference range: PS: A, 60–130%; FPS: Ag, 60–150%. The numbering of changed amino acid residue was numbered by regarding the translation initiation site (start codon) as residue 1. Corresponding PROS1 mutations are exhibited in the bottom of each pedigree.
Supplementary Fig. S1 (Continued)
**Supplementary Fig. S2** The messenger ribonucleic acid (mRNA) analysis of putative splicing site mutation c.1871–2A>G. (A) The sequence diagram of c.1871–2A>G. (B) The sequence diagram of mRNA transcript obtained by TA cloning and sequencing of positive clone. Abbreviations: Del, deletion; E, exon; I, intron. The position of mutated base is indicated with an arrow.
### Supplementary Table S1

Primers used for the amplification of PROS1 exons and intron–exon boundaries

<table>
<thead>
<tr>
<th>Exon (size, bp)</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product (bp)</th>
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<td>CCCTAGGTTCAGAAGCAGAAACA</td>
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Abbreviation: bp, base pair.

### Supplementary Table S2

Results of antibody (LA, ACA or anti-β2 GPI) tests among seven probands with APS

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Abbreviations: ACA, anti-cardiolipin antibody; anti-β2 GPI, anti-β2 glycoprotein I; APS, anti-phospholipid syndrome; IgG/M, immunoglobulin G/M; LA, lupus anticoagulant.

Note: The time space between antibody (LA, ACA or anti-β2 GPI) tests for first time and second time was at least 3 months. LA ≥ 1.2, ACA-IgM ≥ 12 MPL/mL, ACA-IgA ≥ 12 APL/mL, ACA-IgG ≥ 12 GPL/mL or anti-β2 GPI ≥ 20 RU/mL was identified as positive result.
Supplementary Table S3  Clinical manifestations, laboratory examinations and molecular basis of 35 family members with PS deficiency

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<tr>
<th>Family member</th>
<th>Age/sex</th>
<th>Thrombotic episodes and other thrombosis-related symptoms</th>
<th>PS: A (%)</th>
<th>TPS: Ag (%)</th>
<th>FPS: Ag (%)</th>
<th>Type of PS deficiency</th>
<th>Nucleotide variation (DNA) and amino acid substitution</th>
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<td>c.134T&gt;A, p. L45&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>c.200A&gt;C, p. E67A</td>
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<td>37.8</td>
<td>I</td>
<td>E1-E4 deletion&lt;sup&gt;a&lt;/sup&gt;</td>
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(Continued)
**Supplementary Table S3 (Continued)**

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<th>Family member</th>
<th>Age/ sex</th>
<th>Thrombotic episodes and other thrombosis-related symptoms</th>
<th>PS: A (%)</th>
<th>TPS: Ag (%)</th>
<th>FPS: Ag (%)</th>
<th>Type of PS deficiency</th>
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Abbreviations: B, brother (family member column); BL-DVT, bilateral leg DVT; D, daughter (family member column); DVT, deep venous thrombosis; E, exon; F, father (family member column); F, female; FPS: Ag, free protein S antigen; LL-DVT, left leg DVT; M, male; M, mother (family member column); PE, pulmonary embolism; PS: A, protein S activity; RL-DVT, right leg DVT; S, son (family member column); SD, skin discolouration; SI, sister (family member column); SU, skin ulcer; TPS: Ag, total protein S antigen.

Note: Reference range: PS: A, 60–130%; TPS: Ag, 70–150%; FPS: Ag, 60–150%.

*Novel mutation.*
Supplementary Table S4 The peak values of TGT-based TFPI cofactor activity and antigen/activity levels of PS in 21 PS-deficient and 12 normal individuals

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<th>No. of probands</th>
<th>Peak-aPS</th>
<th>Peak + aPS</th>
<th>Peak (%)</th>
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<td>103.6736</td>
<td>43.7</td>
<td>46.7</td>
<td>44.4</td>
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</table>

<table>
<thead>
<tr>
<th>No. of normal controls</th>
<th>Peak-aPS</th>
<th>Peak + aPS</th>
<th>Peak (%)</th>
<th>PS: A (%)</th>
<th>TPS: Ag (%)</th>
<th>FPS: Ag (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>172.91</td>
<td>237.91</td>
<td>137.5918</td>
<td>128.0</td>
<td>138.0</td>
<td>123.0</td>
</tr>
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<td>2</td>
<td>224.56</td>
<td>269.37</td>
<td>119.9546</td>
<td>112.0</td>
<td>121.0</td>
<td>97.0</td>
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<tr>
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<td>205.02</td>
<td>250.00</td>
<td>121.9393</td>
<td>93.0</td>
<td>136.0</td>
<td>107.0</td>
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<td>291.07</td>
<td>128.3264</td>
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<td>148.0</td>
<td>118.0</td>
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<tr>
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<td>127.2899</td>
<td>89.0</td>
<td>104.0</td>
<td>105.0</td>
</tr>
<tr>
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<td>224.67</td>
<td>123.2622</td>
<td>98.0</td>
<td>109.0</td>
<td>98.0</td>
</tr>
<tr>
<td>7</td>
<td>216.52</td>
<td>289.99</td>
<td>133.9322</td>
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<td>123.0</td>
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<tr>
<td>8</td>
<td>198.22</td>
<td>256.54</td>
<td>129.4219</td>
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<td>101.0</td>
</tr>
<tr>
<td>9</td>
<td>177.62</td>
<td>240.12</td>
<td>135.1875</td>
<td>121.0</td>
<td>113.0</td>
<td>120.0</td>
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<td>280.45</td>
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<td>129.0</td>
<td>113.0</td>
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<td>222.61</td>
<td>118.0204</td>
<td>101.0</td>
<td>110.0</td>
<td>104.0</td>
</tr>
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</table>

Abbreviations: aPS, polyclonal antibodies against protein S; FPS: Ag, free protein S antigen; PS: A, protein S activity; TFPI, tissue factor pathway inhibitor; TPS: Ag, total protein S antigen.

Note: The TFPI cofactor activity of PS was described as the ratio of thrombin peaks calculated in the absence and presence of aPS. The number of probands was same as Tables 1 and 2. Reference range: PS: A, 60–130%; TPS: Ag, 70–150%; FPS: Ag, 60–150%.
**Supplementary Table S5** The predicted effects of five novel missense mutations

<table>
<thead>
<tr>
<th>Nucleotide exchange</th>
<th>aa exchange</th>
<th>Domain</th>
<th>SIFT score</th>
<th>PolyPhen-2 score</th>
<th>MutationTaster score</th>
<th>Align GVGD</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.38C&gt;A</td>
<td>Ala13Glu</td>
<td>–</td>
<td>0.06</td>
<td>0.988</td>
<td>0.836</td>
<td>Class C0</td>
<td>Disease causing</td>
</tr>
<tr>
<td>c.721T&gt;C</td>
<td>Cys241Arg</td>
<td>EGF</td>
<td>0.00</td>
<td>1.000</td>
<td>1.000</td>
<td>Class C65</td>
<td>Disease causing</td>
</tr>
<tr>
<td>c.802G&gt;T</td>
<td>Glu268Tyr</td>
<td>EGF</td>
<td>0.05</td>
<td>1.000</td>
<td>1.000</td>
<td>Class C0</td>
<td>Disease causing</td>
</tr>
<tr>
<td>c.1445G&gt;T</td>
<td>Gly482Val</td>
<td>SHBG</td>
<td>0.00</td>
<td>1.000</td>
<td>1.000</td>
<td>Class C65</td>
<td>Disease causing</td>
</tr>
<tr>
<td>c.1915T&gt;G</td>
<td>Cys639Gly</td>
<td>SHBG</td>
<td>0.00</td>
<td>0.999</td>
<td>1.000</td>
<td>Class C65</td>
<td>Disease causing</td>
</tr>
</tbody>
</table>

Abbreviation: aa, amino acid.
Note: SIFT score of < 0.05, PolyPhen-2 score of > 0.80, MutationTaster score of > 0.80 or Align GVGD Class C65 was considered as probably disease causing. Novel missense mutations were identified deleterious when at least two of the four predictions showed pathogenic results.

**Supplementary Table S6** The predicted effects of putative splice site mutation c.1155+5G>C and c.1871–2A>G on normal splicing

<table>
<thead>
<tr>
<th>Nucleotide exchange</th>
<th>Scores predicted by Alamut v2.7</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSF ME NNS GS</td>
<td></td>
</tr>
<tr>
<td>c.1155+5G&gt;C</td>
<td>– – – –</td>
<td>Disease causing</td>
</tr>
<tr>
<td>c.1871–2A&gt;G</td>
<td>– – – –</td>
<td>Disease causing</td>
</tr>
</tbody>
</table>

Abbreviations: GS, GeneSplicer; ME, MaxEntScan; NNS, NNSplicer; SSF, SpliceSiteFinder-like.
Note: The mutation impairing native splice sites were considered to have ‘negative’ effects on splicing. – indicate a great negative effect (the decreased score of the wild-type splice site was higher than 50% of the score of the authentic one). A splice site effect was considered potentially deleterious when at least two of the four predictions showed significant results.