INHERITED THROMBOPHILIA

PCD AS AN EXAMPLE

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WARFARIN-INDUCED SKIN NECROSIS IN PCD PATIENT
WHAT IS PROTEIN C?

- 62-kD vitamin K-dependent glycoprotein
- Synthesized in the liver as a single-chain zymogen
- Clipped into a serine-protease-like enzyme on phospholipid cell surfaces by thrombin
- Protein C also has pro-fibrinolytic, anti-inflammatory and anti-ischemic properties
CLINICAL SIGNIFICANCE

• An increased incidence of *venous thromboembolism* (relative risk 8–10).

• Whereas NO association with *arterial* thrombotic disease has been found.
1960 • Protein C's anticoagulant role in the human body was first noted by Seegers et al. (autoprothrombin II-a)

1976 • Protein C was first isolated by Johan Stenflo from bovine plasma. (Vitamin K-dependent protein)

1977 • It was first recognized that APC inactivates Factor Va
1980
- Vehar and Davie discovered that APC also inactivates Factor VIIIa.
- Protein S was recognized as a cofactor by Walker.

1982
- Griffin discovered the associated protein C deficiency with symptoms of venous thrombosis.

1984
- Homozygous protein C deficiency and the consequent serious health effects were discovered.
cDNA cloning of protein C was first performed by Beckmann et al. which produced a map of the gene responsible for producing protein C in the liver.

Experiment was performed (Taylor et al.) whereby it was demonstrated that APC prevented coagulopathy and death in baboons infused with lethal concentrations of E. coli.

A heritable resistance to APC was detected by Dahlbäck et al. and associated with familial thrombophilia.
2001 • PROWESS clinical trial, it was recognized that many of the symptoms of sepsis may be ameliorated by infusion of APC, and mortality rates of septic patients may be significantly decreased.

2001 • Drotrecogin alfa (activated), a recombinant human activated protein C, became the first drug approved by the U.S. FDA for treating severe sepsis.

2002 • “Science” published an article that first showed protein C activates protease-activated receptor-1 (PAR-1) and this process accounts for the protein's modulation of the immune system.
Protein C gene (PROC) is located on chromosome 2q13-14 consisting of 9 exons and encoding a 1795 bp-mRNA. This mRNA contains the protein c coding region (from exon 2 to 9) and non-translated region.

Each exon in the coding region is responsible for encoding specific structural and/or functional elements of the protein.

More than 370 mutations have been described and linked to PC deficiency (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PROC) accessed 30 August 2016.)
DEFICIENCY OF PROTEIN C AND S

- Congenital PC Deficiency is a rare cause of hereditary thrombophilia implicated only in about 3–5% of all thromboembolic events. (N. Engl. J. Med. 344:1222–1231)
- Congenital PC deficiency is inherited as autosomal dominant disorder with marked variability in the penetrance and phenotypic expression.
- Homozygous and heterozygous forms.
- Homozygous form presents in infancy as neonatal purpura fulminans.
- Heterozygotes generally are not symptomatic until the 3rd and 4th decades.
  - It occurs in 0.14–0.50% of the general population
PCD TYPES

• Type I: quantitative defects of protein C (low production or short protein half life)
• Type II: qualitative defects, in which interaction with other molecules is abnormal:
  • Defects in interaction with thrombomodulin,
  • Phospholipids,
  • Factors V/VIII and others have been described.
• Decreased protein levels
Heterozygous individuals with PC levels around 50% of the reference range have increased risk for venous thrombosis as compared to healthy population.

Those patients can be asymptomatic or might develop thromboembolic events in early adulthood.

The estimated incidence of asymptomatic heterozygous PC deficiency in healthy population is one in 200–500, while the symptomatic form accounts for one in 20,000.
HPCD with very low or undetectable activity level typically manifests in the neonatal period with life-threatening PF and disseminated intravascular coagulation involving eyes, kidneys and CNS.

Ophthalmic manifestations in a form of vitreous hemorrhage, which is due to retinal vessel thrombosis, have been reported and could be the initial presentation.

Neurological complications such as ischemic stroke and hydrocephaly resulting from cerebral vein thrombosis may occur in utero and can be diagnosed antenatally.

It is worth noting that NOT all patients with HPCD will have a full-blown clinical picture in the neonatal period.
## INCIDENCE

<table>
<thead>
<tr>
<th>Category</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>common</strong></td>
<td>G1691A mutation in factor V gene (Leiden mutation)</td>
</tr>
<tr>
<td></td>
<td>G20210A mutation in prothrombin gene</td>
</tr>
<tr>
<td></td>
<td>C677A mutation in MTHFR gene</td>
</tr>
<tr>
<td><strong>Rare</strong></td>
<td>Antithrombin deficiency</td>
</tr>
<tr>
<td></td>
<td>Protein C deficiency (HPCD incidence of 1 per 4 million births)</td>
</tr>
<tr>
<td></td>
<td>Protein S deficiency</td>
</tr>
<tr>
<td><strong>Very rare</strong></td>
<td>Dysfibrinogenemia</td>
</tr>
<tr>
<td></td>
<td>Homozygous homocysteinuria</td>
</tr>
<tr>
<td><strong>Probably inherited</strong></td>
<td>Increase factor VIII, IX, XI or fibrinogen</td>
</tr>
<tr>
<td></td>
<td>Factor VIII and fibrinogen are acute phase</td>
</tr>
</tbody>
</table>

Some reports have described individuals who developed venous thrombosis beyond the first month of life implying that other factors need to be present for full penetrance.

The incidence of homozygous PC deficiency is quite rare with only one case in 500,000 living birth annually.

Some reports have estimated the incidence to be as low as one per 4 million births.
MANAGEMENT

- Protein c concentrate
- Recombinant
- Anticoagulation
- Liver transplantation
Medical care(1)

- Thromboprophylaxis
- Neonatal Warfarin oral suspension.
- VTE:
  - Long-term anticoagulation is often recommended
- Warfarin-induced skin necrosis:
  - Immediate discontinuation of warfarin,
  - Administration of vitamin K,
  - And initiation of therapeutic doses of heparin.
Medical care(2)

• Neonatal purpura fulminans
  • FFP
  • Highly purified protein C concentrate (Ceprotin) represents an attractive alternative that does not subject patients to the high volume and protein load of fresh frozen plasma.

• Living donor liver transplantations have been successfully performed in NPF, resulting in a permanent cure.
A DELAYED PRESENTATION OF HOMOZYGOUS PROTEIN C DEFICIENCY IN A SERIES OF CHILDREN: A REPORT ON TWO MOLECULAR DEFECTS

TO DETERMINE THE GENOTYPE OF FIVE CHILDREN WITH HPCD AND ANALYZE CLINICAL PRESENTATION, PROGNOSIS, AND REVIEW OTHER COMPARABLE RELATED DATA IN THE LITERATURE.
METHODS (1)

- Number = 5 Saudi patients,
  - 2 males
  - 3 females
- Retrospective chart review and during office visits.
- Median age: 2–14 years.
METHODS (2)

• The variables collected include:
  • Age at onset,
  • Gender,
  • Type of presentation,
  • Family,
  • History,
  • Consanguinity,
  • Laboratory data, and
  • Genetic defects.

• Only papers published in English language during the period from 1984-2014 were included.
• Data for 82 patients with HPCD have been reviewed.
METHODS (3)

- Protein C activity was measured by activating protein C present in the patient sample using a specific snake Venom activator.
- The quantitative determination of protein C activity was performed by a chromatographic substrate-Based assay.
- DNA extraction of patient’s leukocytes carried after obtaining an informed consent.
- The PROC gene was then amplified by polymerase Chain reaction (PCR).
- Products of PCR were then Sequenced for both DNA strands of the entire coding region and the highly conserved exon–intron splice junction.
- The genetic studies were carried out in cooperation with the laboratories of Centogene AG and Biocentia, Germany.
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (month)</th>
<th>Site</th>
<th>Purpura fulminans</th>
<th>Eye manifestation</th>
<th>Intracranial manifestation</th>
<th>Molecular defect</th>
<th>Outcome</th>
<th>Other association</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>Thighs, abdomen, chest, and face</td>
<td>1 day</td>
<td>CO</td>
<td>16 month**</td>
<td>(+)</td>
<td>A388V</td>
<td>Blindness</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>Thighs, abdomen, chest, and face</td>
<td>1 day</td>
<td>CO</td>
<td>16 month**</td>
<td>(+)</td>
<td>A388V</td>
<td>Blindness</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>Abdomen, gluteal, and perineum</td>
<td>1 day</td>
<td>RH, CO</td>
<td>1 day</td>
<td>(+)</td>
<td>A388V</td>
<td>Blindness</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>Legs, back, hand, and scalp</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(+)</td>
<td>G433S</td>
<td>No disability</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>Lower limbs</td>
<td>1 day</td>
<td>RD</td>
<td>–</td>
<td>(+)</td>
<td>G433S</td>
<td>Blindness</td>
</tr>
</tbody>
</table>
MOLECULAR DEFECTS IN PROTEIN C GENE SAMPLED FROM FIVE CHILDREN WITH HPCD AND THEIR BIOLOGICAL PARENTS

<table>
<thead>
<tr>
<th>Case</th>
<th>State</th>
<th>Nucleotide sequence</th>
<th>Amino acid sequence</th>
<th>Paternal data</th>
<th>Mother data</th>
<th>Consanguinity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Father</td>
<td>Mother</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Homozygous</td>
<td>E9: c.1163 C&gt;T</td>
<td>Ala388Val</td>
<td>70 Heterozygous*</td>
<td>40 Heterozygous*</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Homozygous</td>
<td>E9: c.1163 C&gt;T</td>
<td>Ala388Val</td>
<td>40 Heterozygous*</td>
<td>49 Heterozygous*</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Homozygous</td>
<td>E9: c.1163 C&gt;T</td>
<td>Ala388Val</td>
<td>40 Heterozygous*</td>
<td>49 Heterozygous*</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Homozygous</td>
<td>E9: c.1297 G&gt;A</td>
<td>Gly433Ser</td>
<td>57 Heterozygous*</td>
<td>42 Heterozygous*</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Homozygous</td>
<td>E9: c.1297 G&gt;A</td>
<td>Gly433Ser</td>
<td>NR Heterozygous*</td>
<td>NR Heterozygous*</td>
<td>+</td>
</tr>
</tbody>
</table>

PCa, protein C activity; NR, not recorded.

*Heterozygous for the same mutation seen in his/her offspring.
LABORATORY DATA OF FIVE SAUDI CHILDREN WITH HOMOZYGOUS PROTEIN C DEFICIENCY AT PRESENTATION

<table>
<thead>
<tr>
<th>Case</th>
<th>PLT (*10^9)</th>
<th>PT (sec)</th>
<th>PTT (sec)</th>
<th>INR</th>
<th>Fibrinogen (g/L)</th>
<th>D-dimer (mg/L FEU)</th>
<th>PC activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>18</td>
<td>200</td>
<td>1.6</td>
<td>0.4</td>
<td>35.9</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>17</td>
<td>44</td>
<td>1.4</td>
<td>0.4</td>
<td>35.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>15</td>
<td>45</td>
<td>1.4</td>
<td>0.6</td>
<td>6.4</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>180</td>
<td>15</td>
<td>50</td>
<td>1.3</td>
<td>2.8</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>222</td>
<td>16</td>
<td>45</td>
<td>1.3</td>
<td>0.6</td>
<td>0.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal values: PLT = 150–450 *10^9, INR = 0.9–1.2, PT = 11–17 sec, PTT = 28–42 sec, fibrinogen = 0.5–4 g/L, D-dimer = <0.5, ESR = 0–10.
## COMPARISON

<table>
<thead>
<tr>
<th></th>
<th>N = 5 (%)</th>
<th>N = 82 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>5 (100)</td>
<td>53 (65)</td>
</tr>
<tr>
<td>Ocular findings:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Blindness</td>
<td>4 (80)</td>
<td>16 (20)</td>
</tr>
<tr>
<td>• Retinal Detachment</td>
<td>1 (20)</td>
<td>10 (12)</td>
</tr>
<tr>
<td>IC</td>
<td>3 (60)</td>
<td>8 (10)</td>
</tr>
</tbody>
</table>

**Ocular findings:**
- Blindness
- Retinal Detachment
MESSAGES

• Pcd is a rare inherited thrombophilia.

• Different genotypic and phenotypic presentation

• Considering the rarity and potentially irreversible outcomes of HPCD, prompt recognition and timely intervention can highly impact on the prognosis
REFERENCES


