Heterozygous mutations in PLCZ1 are associated with fertilization failure after ICSI

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Introduction: PLCZ1 is a crucial sperm factor required for oocyte activation
Mutations in PLCZ1 are associated with failed fertilization after ICSI


To date 11 different mutations in PLCZ1 gene are reported in 22 patients with OAD
Diagnostic tests to evaluate the patient sperm activation capacity

**Mouse Oocyte Activation Test (MOAT)**

- Patient sperm (sample to test)
- Piezo-driven ICSI
- MOAT result = % of 2-cell embryos
  - MOAT group 1 (0-20%)
  - MOAT group 2 (21-84%)
  - MOAT group 3 (85-100%)

**Mouse Oocyte Calcium Analysis (MOCA)**

- Patient sperm (sample to test)
- Piezo-driven ICSI
- Fresh MII mouse oocytes
- MOCA result = A x F score
  - AxF >9
  - AxF <9

**Human Oocyte Calcium Analysis (HOCA)**

- Patient sperm (sample to test)
- ICSI
- In vitro matured control human oocytes
- HOCA result = A x F score
  - AxF >1
  - AxF <1
Partial hydatidiform moles (PHMs) and abnormal calcium oscillations

Sperm involved in recurrent partial hydatidiform moles cannot induce the normal pattern of calcium oscillations

Dimitra Nikiforaki, M.Sc., a Frauke Vanden Meerschaut, M.D., Ph.D., a Stefanie De Gheselle, M.Sc., a Chen Qian, M.D., a Etienne Van den Abbeel, Ph.D., a Winnok Harald De Vos, Ph.D., b,c Tom Deroo, Ph.D., a Petra De Sutter, M.D., Ph.D., a and Björn Heindryckx, Ph.D. a
Partial Hydatidiform Mole

- Partial hydatidiform mole is a gestational trophoblastic disease with trophoblastic proliferation of chorionic villi and no embryonic development.

Chorionic villi

Triploid Dispermic

= maternal chromosome

= paternal chromosome
Mechanism for the origin of triploid PHMs

Dispermy
- 90%
  - 23X or Y

Diploid sperm
- 10%
  - 46XX or XY

Ovum
- 23X
- 69XXX
- 69XXY
- 69XYY

Possible mechanisms for the formation of triploid Paral moles
Objectives

- To screen patients with fertilization failure after ICSI for mutations in PLCZ1 gene
- To screen patients with PHMs for mutations in PLCZ1 gene
- Functional testing of the sperm using mouse and human oocytes:
  - Mouse Oocyte Activation Test (MOAT)
  - Mouse oocyte calcium analysis (MOCA)
  - Human Oocyte Calcium Analysis (HOCA)
Materials and Methods: patient recruitment

- 38 couples with low (<33%) or total fertilization failure after ICSI were recruited in the study.
- Mouse oocyte activation test on the spermatozoa revealed:
  - 35 patients: mostly MOAT 1&2
  - 3 PHM patients: 1 MOAT 2 /2 MOAT3

<table>
<thead>
<tr>
<th>MOAT Group</th>
<th>Percentage Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOAT 1</td>
<td>0-20%</td>
</tr>
<tr>
<td>MOAT 2</td>
<td>21-84%</td>
</tr>
<tr>
<td>MOAT 3</td>
<td>85-100%</td>
</tr>
</tbody>
</table>
Materials and Methods: *PLCZ1* sequencing

- Saliva sample collection with Oragene saliva kit
- DNA quality and quantity assessment
- DNA extraction and purification
- PCR amplification and fragment analysis
- DNA extraction and purification
- Qubit
- Miseq and Sanger sequencing
Materials and Methods: Functional test

- Functional test of the identified mutations by calcium analysis

Mouse Oocyte Calcium Analysis
AXF ≤ 9

Human Oocyte Calcium Analysis
AXF ≤ 1
Results: *PLCZ1* sequencing

<table>
<thead>
<tr>
<th>Code</th>
<th>MOAT</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Mutation type</th>
<th>ExAC frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (PHM)</td>
<td>89%</td>
<td>c.136G&gt;A; c.1499C&gt;T</td>
<td>p.D46N; p.S500L</td>
<td>Compound Heterozygous</td>
<td>0.00002; 0.03</td>
</tr>
<tr>
<td>P2 (PHM)</td>
<td>78%</td>
<td>c.136-1 G&gt;C</td>
<td>p.?</td>
<td>Heterozygous</td>
<td>Novel</td>
</tr>
<tr>
<td>P3 (PHM)</td>
<td>97%</td>
<td>c.698A&gt;T</td>
<td>p. H233L</td>
<td>Heterozygous</td>
<td>0.0007</td>
</tr>
<tr>
<td>P4</td>
<td>19%</td>
<td>c.698A&gt;T; c.964A&gt;T</td>
<td>p. H233L; p.K322*</td>
<td>Compound heterozygous</td>
<td>0.0007; Novel</td>
</tr>
<tr>
<td>P5</td>
<td>78%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Heterozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P6</td>
<td>40%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Heterozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P7</td>
<td>84%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Heterozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P8</td>
<td>83%</td>
<td>c.422G&gt;A</td>
<td>p. R141H</td>
<td>Heterozygous</td>
<td>0.0002</td>
</tr>
<tr>
<td>P9</td>
<td>50%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Heterozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P10</td>
<td>54%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Heterozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P11</td>
<td>64%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Heterozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P12</td>
<td>89%</td>
<td>c.698A&gt;T</td>
<td>p. H233L</td>
<td>Heterozygous</td>
<td>0.0007</td>
</tr>
<tr>
<td>P13</td>
<td>79%</td>
<td>c.1499C&gt;T</td>
<td>p.S500L</td>
<td>Homozygous</td>
<td>0.03</td>
</tr>
<tr>
<td>P14</td>
<td>89%</td>
<td>c.280C&gt;T; c.1499C&gt;T</td>
<td>p.Q94*, p.S500L</td>
<td>Compound heterozygous</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Identified seven different mutations in 14 of the 38 patients with OAD = 37%
Results: Validation using sanger sequencing

- c.136 G>A; D46N
- c.136 -1 G>C
- c.698A>T; H233L
- c.422G>A; R141H
- c.1499C>T; S500L
- c.689A>T; K322*
- c.280C>T; Q94*
Results: Functional Analysis MOCA and HOCA

<table>
<thead>
<tr>
<th>Patient</th>
<th>MOAT</th>
<th>MOCA (AXF)</th>
<th>HOCA (AXF)</th>
<th>MOCA</th>
<th>HOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4 (H233L; K322*)</td>
<td>19%</td>
<td>2 (17)</td>
<td>0 (10)</td>
<td>Control</td>
<td>75.2 (3)</td>
</tr>
<tr>
<td>P7 (S500L)</td>
<td>84%</td>
<td>2.04 (11)</td>
<td>0 (8)</td>
<td>Control</td>
<td>42.4 (2)</td>
</tr>
<tr>
<td>P8 (R141H)</td>
<td>83%</td>
<td>8.02 (12)</td>
<td>0 (7)</td>
<td>Control</td>
<td>42.4 (2)</td>
</tr>
<tr>
<td>P10 (S500L)</td>
<td>54%</td>
<td>35 (11)</td>
<td>0.25 (14)</td>
<td>Control</td>
<td>48.3 (3)</td>
</tr>
<tr>
<td>P11 (S500L)</td>
<td>64%</td>
<td>12.4 (11)</td>
<td>0 (10)</td>
<td>Control</td>
<td>48.3 (3)</td>
</tr>
<tr>
<td>P12 (H233L)</td>
<td>89%</td>
<td>13.0 (10)</td>
<td>0 (13)</td>
<td>Control</td>
<td>48.3 (3)</td>
</tr>
<tr>
<td>P13 (S500L)</td>
<td>79%</td>
<td>15.8 (11)</td>
<td>0 (9)</td>
<td>Control</td>
<td>42.4 (2)</td>
</tr>
</tbody>
</table>

MOCA and HOCA analysis was performed on the sperm of 7 patients with OAD after ICSI

Almost all the patient sperm showed no calcium oscillations when injected in to human oocytes.
Results: MOCA and HOCA analysis on the sperm causing PHMs

<table>
<thead>
<tr>
<th>Patient</th>
<th>MOAT</th>
<th>MOCA (AXF)</th>
<th>HOCA (AXF)</th>
<th>MOCA</th>
<th>HOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (D46N; S500L)</td>
<td>89%</td>
<td>5.89 (20)</td>
<td>0.01 (9)</td>
<td>Control</td>
<td>42.4 (2)</td>
</tr>
<tr>
<td>P2 (C.136-1G&gt;C)</td>
<td>78%</td>
<td>4.36 (15)</td>
<td>0 (18)</td>
<td>Control</td>
<td>48.3 (3)</td>
</tr>
<tr>
<td>P3 (H233L)</td>
<td>97%</td>
<td>4.17 (16)</td>
<td>0.69 (9)</td>
<td>Control</td>
<td>46.4 (4)</td>
</tr>
</tbody>
</table>

**HOCA with single sperm injection**

- **Ca\(^{2+}\) oscillations**
  - P1 (18): 17% +++
  - P2 (18): 0% +
  - P3 (9): 44% ++
- **No oscillations**
  - Control: 91.2% 63.2%
  - P1 (18): 83% 14.0%
  - P2 (18): 100% 14.0%
  - P3 (9): 56% 8.8%
Results: HOCA analysis after two sperm injection from the patient sperm causing PHMs

<table>
<thead>
<tr>
<th>Patient</th>
<th>HOCA (AXF) with two sperm injection</th>
<th>HOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (D46N; S500L)</td>
<td>0.8 (20)</td>
<td>Control 6.96 (2)</td>
</tr>
<tr>
<td>P2 (C.136-1G&gt;C)</td>
<td>0 (10)</td>
<td>Control 5.99 (4)</td>
</tr>
</tbody>
</table>
Hypothesis for the formation of PHMs in patients with PLCZ1 mutations

PLCZ1 mutation

PLCZ1 mutation + WT PLCZ1

No activation

PHM
Summary

- Screening of 38 patients with failed fertilization after ICSI for mutations in PLCZ1 has led to the identification of 7 different mutations in 14 patients (37%)

- Three of the patients had recurrent PHMs suggesting a role of PLCZ1 in the causation of PHMs

- HOCA using single and two sperm injections on the human oocytes showed that the two injection with PLCZ1 mutations increased the number of oocytes getting activated

- This is the first study to show a paternal genetic link in causing PHMs
Cooperations

UGent:
D. Deforce/F. Van Nieuwerburgh
A. Van Soom/L. Peelman
L. Leybaert
B. Menten/P. Coucke/
J. VandeSompele/E. De Baere
R. Van Coster

International:
S. Chuva de Sousa Lopes (Leiden)
K. Coward (Oxford)
J. Parrington (Oxford)
Thank you for your attention