Whole-exome sequencing reveals defective \textit{CYP3A4} variants predictive for paclitaxel dose-limiting neuropathy

\textbf{María Apellániz Ruiz}
Spanish National Cancer Research Centre (CNIO)
May 19th 2014
Paclitaxel

• **Microtubule binding drug**, effective for many solid tumors (breast, ovarian, lung)
• **Dose limiting toxicity**: **peripheral neuropathy** (30-50% of patients affected)
• Paclitaxel **dose reduction/treatment suspension** in 10% patients
• **Large inter-individual variability**, some cases irreversible nerve damage
• No effective treatment to prevent or treat neuropathy

β tubulin subunit

α tubulin subunit

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Genetic markers of paclitaxel neuropathy

GWAS meta-analysis
  \[ EPHAS5 \rightarrow P=1.4 \times 10^{-9} \]
  \[ XKR4 \rightarrow P=3.1 \times 10^{-8} \]

- Leandro-Garcia, Clin Cancer Res (2012), P=0.021

CYP2C8
- Leskelä, Pharmacogenomics J (2011), P=0.032
- Hertz, Ann Oncol (2013), P=0.006

CYP3A4
- de Graan, Clin Cancer Res (2013), P=0.001

ABCB1
- Sissung, Eur J Cancer (2006), P=0.09
**Genetic markers of paclitaxel neuropathy**

**Peripheral nervous system**

- SLCO1B3
- ABCB1
- GWAS meta-analysis
  - EPHA5 $\rightarrow$ P=1.4 x 10^{-9}
  - XKR4 $\rightarrow$ P=3.1 x 10^{-8}

**Common variants (moderate effects)**

- CYP2C8
  - Leskelä, *Pharmacogenomics J* (2011), P=0.032
  - Hertz, *Ann Oncol* (2013), P=0.006

- CYP3A4
  - de Graan, *Clin Cancer Res* (2013), P=0.001

- ABCB1
  - Sissung, *Eur J Cancer* (2006), P=0.09
However, a large part of inter-individual differences in paclitaxel neuropathy susceptibility remain unexplained

**Objective:**

Identify genetic markers predictive of paclitaxel neurotoxicity, taking into account low-frequency & rare variants
Extreme phenotype approach coupled with WES

Paclitaxel neuropathy

Nr individuals
Extreme phenotype approach coupled with WES

- Rare variants (strong effects)
  - Bottom phenotype

- Common variants

- Rare variants (strong effects)
  - Top phenotype

Paclitaxel neuropathy
Extreme phenotype approach coupled with WES

Rare variants (strong effects)

Common variants

Rare variants (strong effects)

Bottom phenotype

Top phenotype

Paclitaxel neuropathy

n=8 patients
Extreme phenotype approach coupled with WES

Whole exome sequencing (WES)
**Characteristics of the patients with extreme neuropathy**

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*Confounders ruled out (diabetes, alcohol intake, previous neuropathies..)*
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Whole exome sequencing (WES)

DNA

Peripheral blood samples (n=8)

Variant Filtering

- Genome regions with low mappability
- With strand bias p-value < 0.001 in at least one sample
  - With low depth read (<15x)
- Alternative allele present in <20% of reads

SNVs and Indels

- SureSelect XTHumanAllExon 50Mb
- HiSeq2000
- Median coverage 50x
Whole exome sequencing (WES)

Peripheral blood samples (n=8)

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SNVs and Indels

Variants in genes paclitaxel PD/ PK
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Rare *CYP3A4* high-impact variants in extreme neuropathy patients

**Indel: Nonsense variant**

P488Tfs*494 (CYP3A4*20)

**SNV: Missense variant**

P389S

Wild type
Rare *CYP3A4* high-impact variants in extreme neuropathy patients

**Indel: Nonsense variant**

P488Tfs*494 (CYP3A4*20)

![Wild type](image1)

**SNV: Missense variant**

P389S

![Wild type](image2)

---

**CYP3A4 wt**

GGACTTCTTCAACCAGAAAAACCCGGTTCTAAAGGTGAGTCAAGGGATGCGACCGTAAGTGGAAGCCTGA

GLLQPPEKPVYLKVESRDRGTVSQAG

**CYP3A4*20**

GGACTTCTTCAACCAGAAAAACCCGGTTCTAAAGGTGAG

GLLQPPEKTCSKG*
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- **Indel: Nonsense variant**
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- Westlind-Johnsson *et al* 2006
  - Protein devoid of functional activity
  - 1 volunteer with a 6-fold higher exposure of CYP3A4 substrate
  - Not found in 428 Germans

- Rare variant (EVS:9 carriers in 6500 individuals)
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Rare variant (EVS: 9 carriers in 6500 individuals)

Westlind-Johnsson et al 2006

• Non-described
• Conserved β-helix
• Defective (4/4 software prediction)

SNV: Missense variant

P389S

Wild type
Screening for **CYP3A4** coding variants

- Breast/ovarian tumors
- 97% first line paclitaxel
- Full neuropathy data

**CYP3A4** coding region
DHPLC screening

- 3 carriers *CYP3A4*20
- 1 carrier of novel L475V
- 1 carrier (R130Q) *CYP3A4*8

N = 228 patients
## Screening for CYP3A4 coding variants

### Summary of CYP3A4 variants

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<td></td>
<td>*20</td>
<td>DHPLC</td>
<td>3</td>
<td>8</td>
<td>Red (cycle 4)</td>
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<td></td>
<td>*20</td>
<td>DHPLC</td>
<td>3</td>
<td>12</td>
<td>Red (cycle 9)</td>
<td>(6)</td>
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<td>16</td>
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<tr>
<td>5</td>
<td>Decreased activity</td>
<td>P389S (*25)</td>
<td>WES</td>
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<td>6</td>
<td>Susp (cycle 6)</td>
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<tr>
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<td>R130Q (*8)</td>
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<tr>
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<td>L475V (*26)</td>
<td>DHPLC</td>
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<td>8</td>
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<td>(19)</td>
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</tbody>
</table>

### Summary of CYP3A4 variants

<table>
<thead>
<tr>
<th>Patient</th>
<th>CYP3A4 variant effect</th>
<th>CYP3A4 variant allele</th>
<th>Detection technique</th>
<th>Max. Neuropathy grade</th>
<th>Total nr. cycles</th>
<th>Treatment modifications</th>
<th>Time with neuropathy (months)</th>
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<tbody>
<tr>
<td>1</td>
<td>Loss-of-function</td>
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<td>WES</td>
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<td>Red (cycles 4 &amp; 8)</td>
<td>(35)</td>
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<td>*20</td>
<td>DHPLC</td>
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Functional characterization (*25 & *26)

Mi-Young Lee
Magnus Ingelman-Sundberg
(Abstract P-187)
Are CYP3A4 defective variants clinically relevant?

2-fold higher risk of Grade 3 neuropathy

Proportion of patients

<table>
<thead>
<tr>
<th>CYP3A4 Loss-of-function (n=4)</th>
<th>CYP3A4 Missense (n=2)</th>
<th>CYP3A4 Wild type (n=229)</th>
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<tbody>
<tr>
<td>Grade 3</td>
<td>Grade 2</td>
<td>Grade 1</td>
</tr>
<tr>
<td>Grade 0</td>
<td>Grade 0</td>
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</tbody>
</table>

P=0.042

Correction for neuropathy risk factors or tumor type does not change association
Are CYP3A4 defective variants clinically relevant?

2-fold higher risk of Grade 3 neuropathy

7-fold higher risk of treatment modifications

P=2.6x10^-5

Correction for neuropathy risk factors or tumor type does not change association
Conclusions

• Patients carrying CYP3A4 loss of function variants (CYP3A4*20 allele) have increased risk of paclitaxel-induced neuropathy and higher risk of treatment modifications
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• As expected, carriers of missense variants showed an intermediate phenotype concordant with a decreased but not abolished *CYP3A4* activity.
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- These variants may be potential markers for paclitaxel treatment individualization.
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- First description of a genetic marker predictive of paclitaxel dose-limiting toxicity.

- These variants may be potential markers for paclitaxel treatment individualization.

- Low-frequency/rare variants may explain part of CYP3A4 variability and could contribute to adverse drug reactions, specially for narrow therapeutic index drugs.
Acknowledgements

Spanish National Cancer Research Centre (CNIO)  
Hereditary Endocrine Cancer Group

Isabel Calvo  
Laura Gª Estévez  
Jesús García-Donás

Gerardo Gutiérrez  
Maria Sereno  
Eva Guerra  
Óscar Pastor

Beatriz Castelo  
Andrés Redondo

Magnus Ingelman-Sundberg  
Mi-Young Lee  
Souren Mkrtchian  
Inger Johansson

Founding

Isabel Calvo
Laura Gª Estévez
Jesús García-Donás

Gerardo Gutiérrez
Maria Sereno
Eva Guerra
Óscar Pastor

Beatriz Castelo
Andrés Redondo
• **Effective therapy** for many solid tumors (breast, ovarian, lung, pancreas)

• Dose limiting toxicity: *peripheral neuropathy*

**Paclitaxel**

Genetic markers of paclitaxel neuropathy

**I.V.**

**Plasma**

**Paclitaxel**

- **CYP2C8**
  - Leskelä, *Pharmacogenomics J* (2011), P=0.032
  - Hertz, *Ann Oncol* (2013), P=0.006

- **CYP3A4**
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- **ABCB1**
  - Sissung, *Eur J Cancer* (2006), P=0.09

**GWAS metanalysis**


  - **EPHAS** \( P=1.4 \times 10^{-9} \)
  - **XKR4** \( P=3.1 \times 10^{-8} \)

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**Genetic markers of paclitaxel neuropathy**

**SLCO1B3**
- Paclitaxel
- Plasma
- Hepatocyte

**Paclitaxel**
- I.V.

**CYP3A4**
- Paclitaxel
- 3'OH PAC
- diOH PAC

**CYP2C8**
- Paclitaxel
- 6aOH PAC

**ABCB1**
- ABCB1

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---

**Diagram:**
- **Paclitaxel** administered I.V.
- **Plasma**:
  - **Paclitaxel**
  - **SLCO1B3**
  - **CYP3A4**
  - **CYP2C8**
  - **CYP2C8**
  - **ABCB1**
- **Hepatocyte**:
  - **3´OH PAC**
  - **6aOH PAC**
  - **diOH PAC**
- **Biliar canaliculi**
Genetic markers of paclitaxel neuropathy

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Genetic markers of paclitaxel neuropathy

Peripheral nervous system

Paclitaxel

I.V.

Plasma

Paclitaxel

3’OH PAC

diOH PAC

6aOHPAC

SLCO1B3

CYP3A4

CYP2C8

CYP2C8

XKR4

EPHA5

GWAS metanalysis


- CYP2C8
  - Leskelä, Pharmacogenomics J (2011), P=0.032
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  - de Graan, Clin Cancer Res (2013), P=0.001

- ABCB1
  - Sissung, Eur J Cancer (2006), P=0.09
Genetic markers of paclitaxel neuropathy: genome wide approaches (GWAS)

- Balwin *Clin Cancer Res* (2012)
  - Human 610k
  - Paclitaxel 175mg/m², every 14 d
  - Breast cancer, n=855
  - Accumulated Pac dose to G2 neuropathy

  - Human 660WQuad
  - Paclitaxel 175mg/m² + Carboplatin 5-6 AUC, every 21 d
  - Mainly ovarian cancer, n=144
  - Accumulated Pac dose to G2 neuropathy

Meta-analysis top hits

**EPHA5** (rs7349683): HR=1.7, \( P=1.4 \times 10^{-9} \) (1x10^{-6}; 3x10^{-4}), MAF=0.38

**XKR4** (rs4737264): HR=1.7, \( P=3.1 \times 10^{-8} \) (2x10^{-6}; 3x10^{-3}), MAF=0.23
Genetic markers of paclitaxel neuropathy: candidate gene approaches

**Genetic risk factors:** common variants with moderate effects (HR=1.5-2.0)

- **CYP2C8**
  - Leskelä, *Pharmacogenomics J* (2011)

- **CYP3A4**

- **ABCB1**

- **TUBB2A**

**Pharmacokinetics**

- **CYP2C8**
  - *CYP2C8*3
    - RISK P=0.032
    - RISK P=0.006 (MAF=0.11)

- **CYP2C8 Haplotype C**
  - PROTECTION P=0.014, (MAF=0.22)

- **CYP3A4**
  - *CYP3A4*22
    - RISK P=0.001, (MAF=0.05)

- **ABCB1**
  - 3435C>T
    - RISK P=0.09, (MAF=0.47)

**Pharmacodynamics**

- **TUBB2A**
  - -101 &-112 variants
    - PROTECTION P=0.021, (MAF=0.29)
Do patients with *CYP3A4* defective alleles have functional variants previously associated with neuropathy?

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant name</th>
<th>Nr. of neuropathy risk alleles</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
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<td>0</td>
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</table>

**CYP3A4 coding variants**

- Loss-of-function (no activity)
- Missense (decreased activity)

**Paclitaxel-induced neuropathy**

(grade/ dose modif.)

- 3/ Red (2)
- 3/ Red
- 3/ Red
- 3/ -
- 3/ Susp
- 3/ -
- 1/ -
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2-fold higher risk of Grade 3 neuropathy

Proportion of patients

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Correction for neuropathy risk factors or tumor type does not change association
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- CYP3A4 Wild type (n=229)

P=0.042

7-fold higher risk of treatment modifications

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P=5.9x10^{-5}

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<tbody>
<tr>
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Proportion of patients

<table>
<thead>
<tr>
<th>Grade 3</th>
<th>Grade 2</th>
<th>Grade 1</th>
<th>Grade 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>40%</td>
<td>20%</td>
<td>40%</td>
<td>0%</td>
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P=5.8x10^{-3}