Whole-exome sequencing reveals defective *CYP3A4* variants predictive for paclitaxel dose-limiting neuropathy

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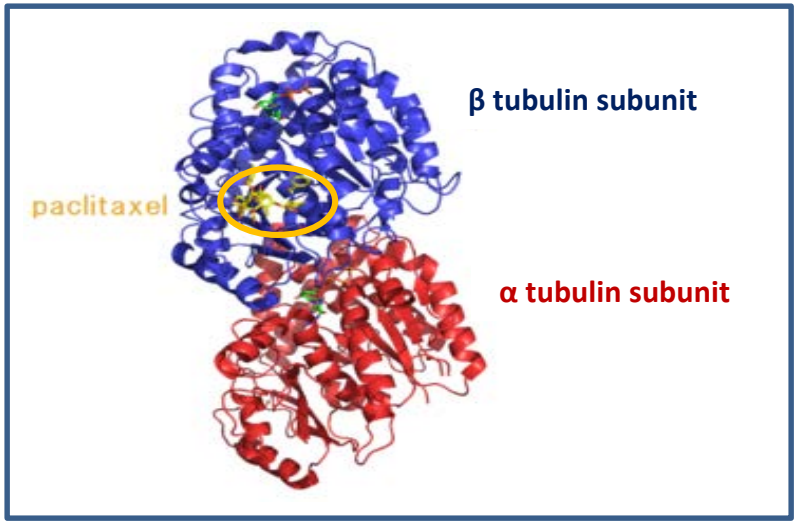
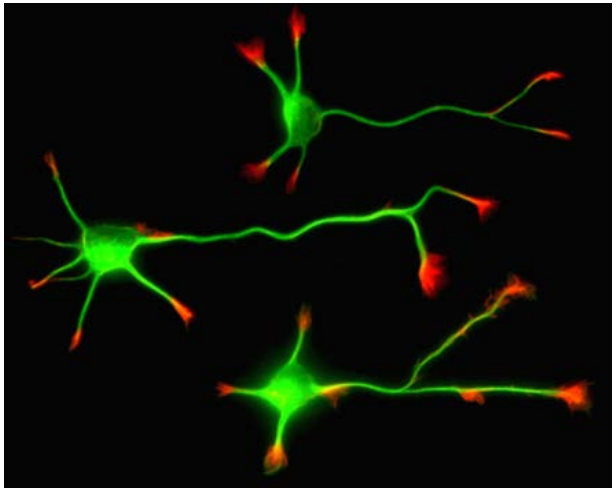
Paclitaxel

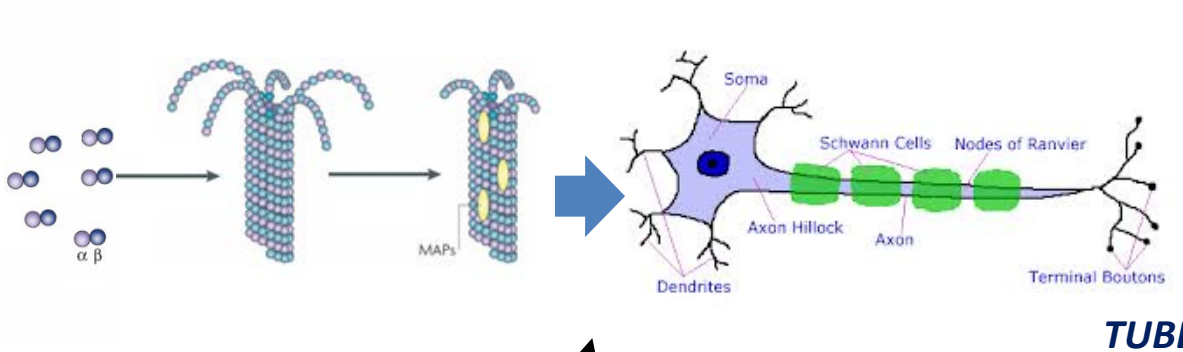
- **Microtubule binding drug**, effective for many solid tumors (breast, ovarian, lung)
- **Dose limiting toxicity**: **peripheral neuropathy** (30-50% of patients affected)
- Paclitaxel **dose reduction/ treatment suspension** in 10% patients
- **Large inter-individual variability**, some cases irreversible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





Wani et al. *J Am Chem Soc. (*1971); Schiff et al. *Nature* (1979); Kuroi & Shimozuma. *Breast Cancer* (2004); Argyriou et al. *Crit Rev Oncol Hematol (2008);* Balayssac et al. *Expert Opin Drug Saf* (2011)

Genetic markers of paclitaxel neuropathy



GWAS meta-analysis

-Balwin, *Clin Cancer Res* (2012)

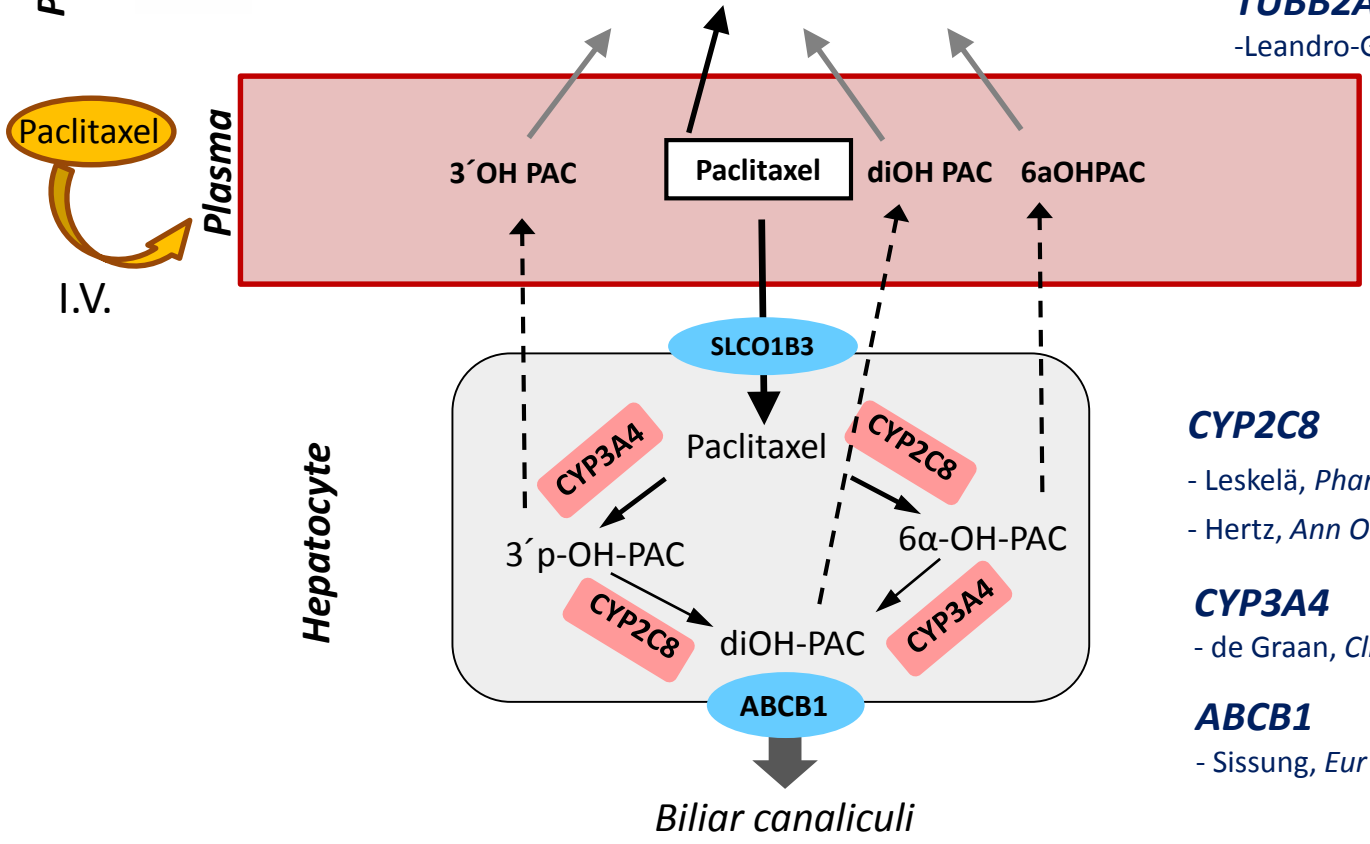
-Leandro-Garcia, *J Med Genet* (2013)

EPHA5 → P=1.4 x 10^{-9}

XKR4 → P=3.1 x 10^{-8}

TUBB2A

-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

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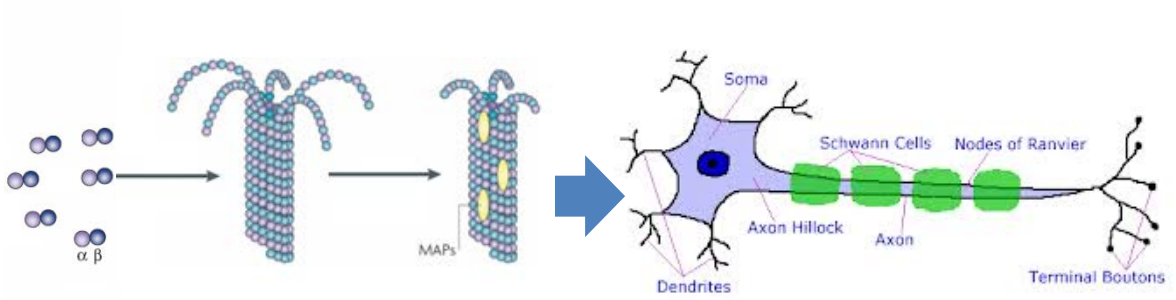

Peripheral nervous system

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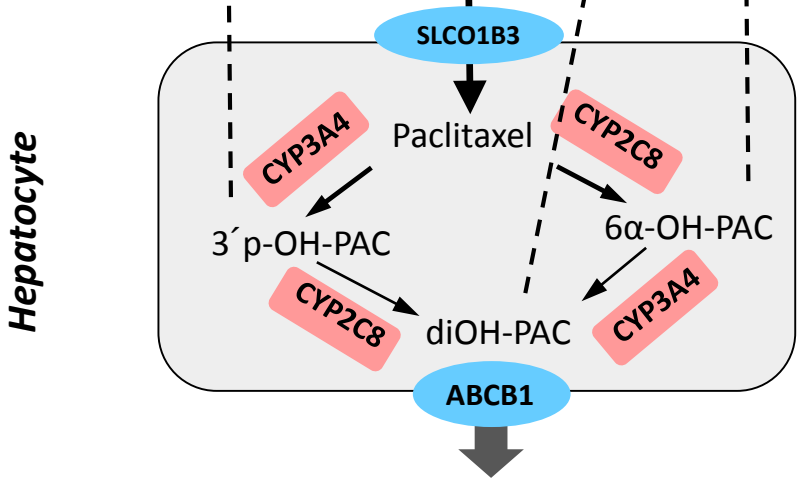
TUBB2A

21

Common variants (moderate effects)

Pacl

I.V.



Hepatocyte

Biliar canaliculi

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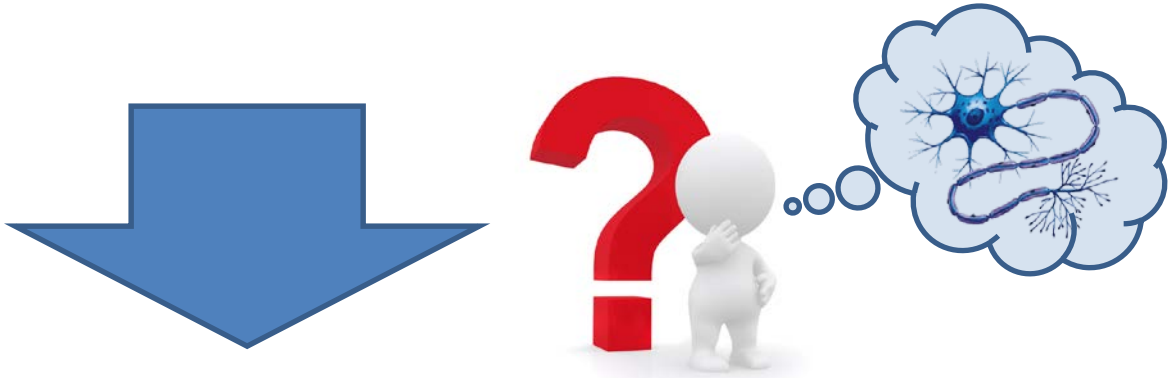
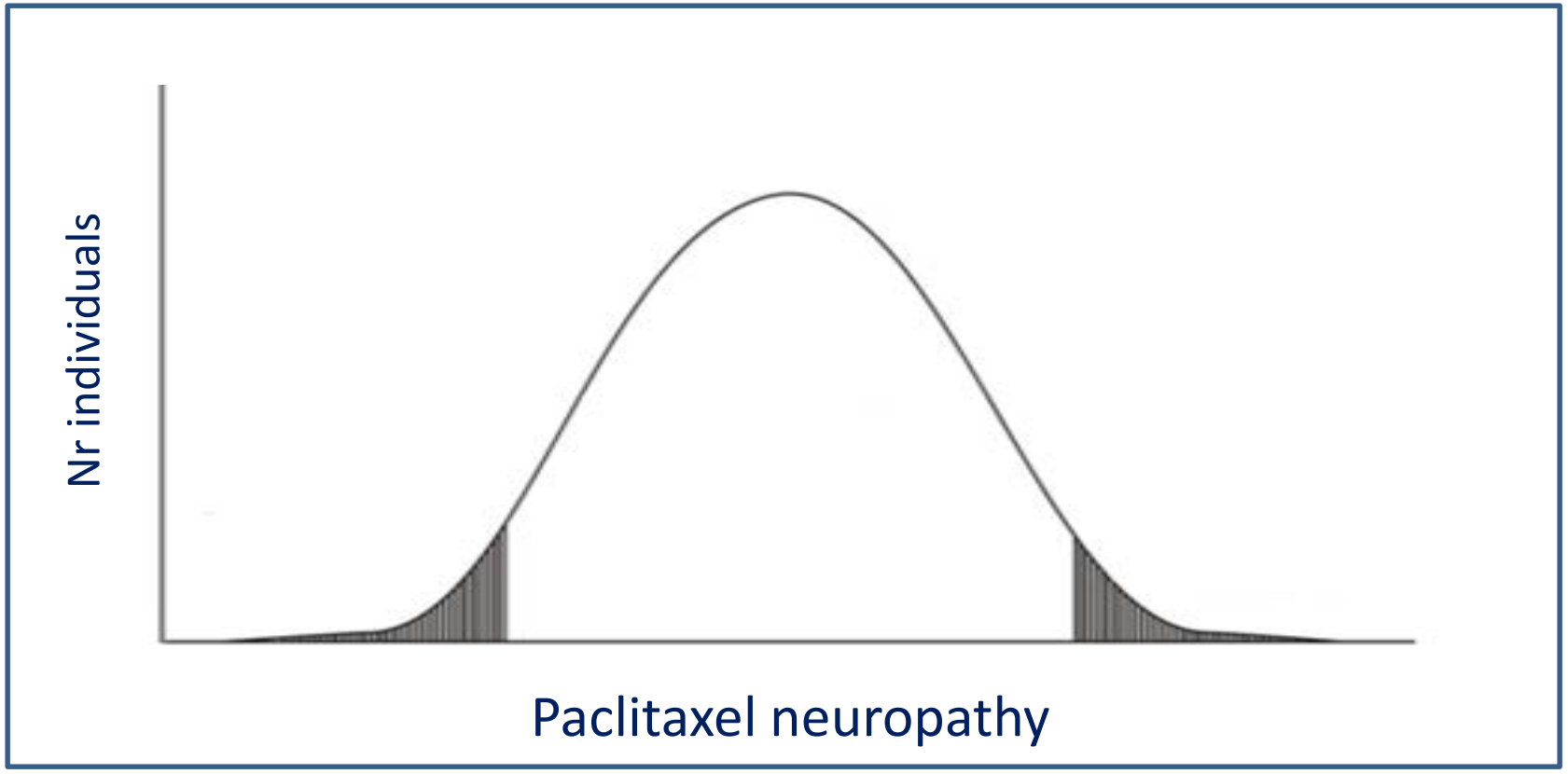
However, a large part of inter-individual differences in paclitaxel neuropathy susceptibility remain unexplained



Objective:

Identify genetic markers predictive of paclitaxel neurotoxicity, taking into account low-frequency & rare variants

Extreme phenotype approach coupled with WES



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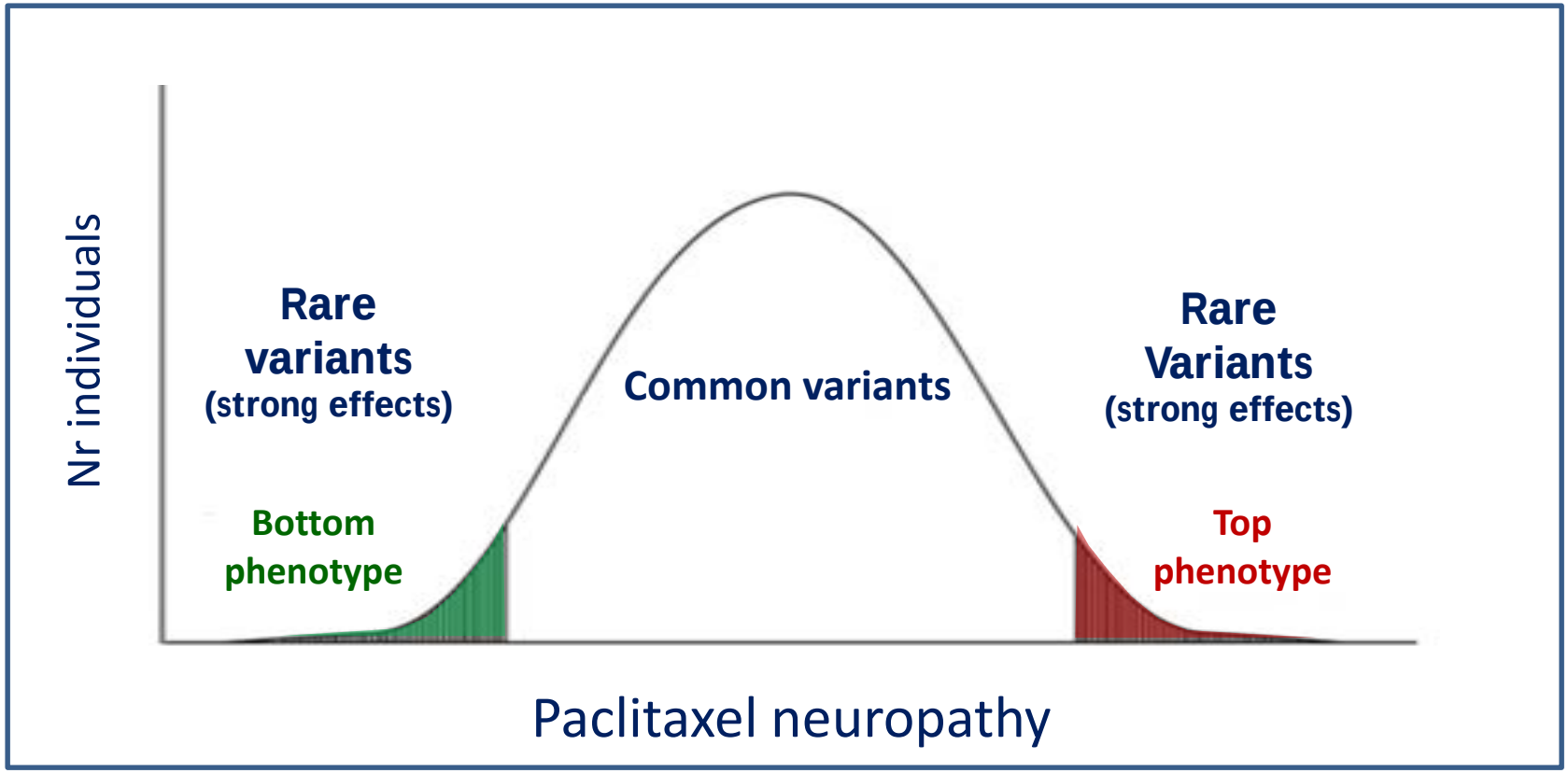

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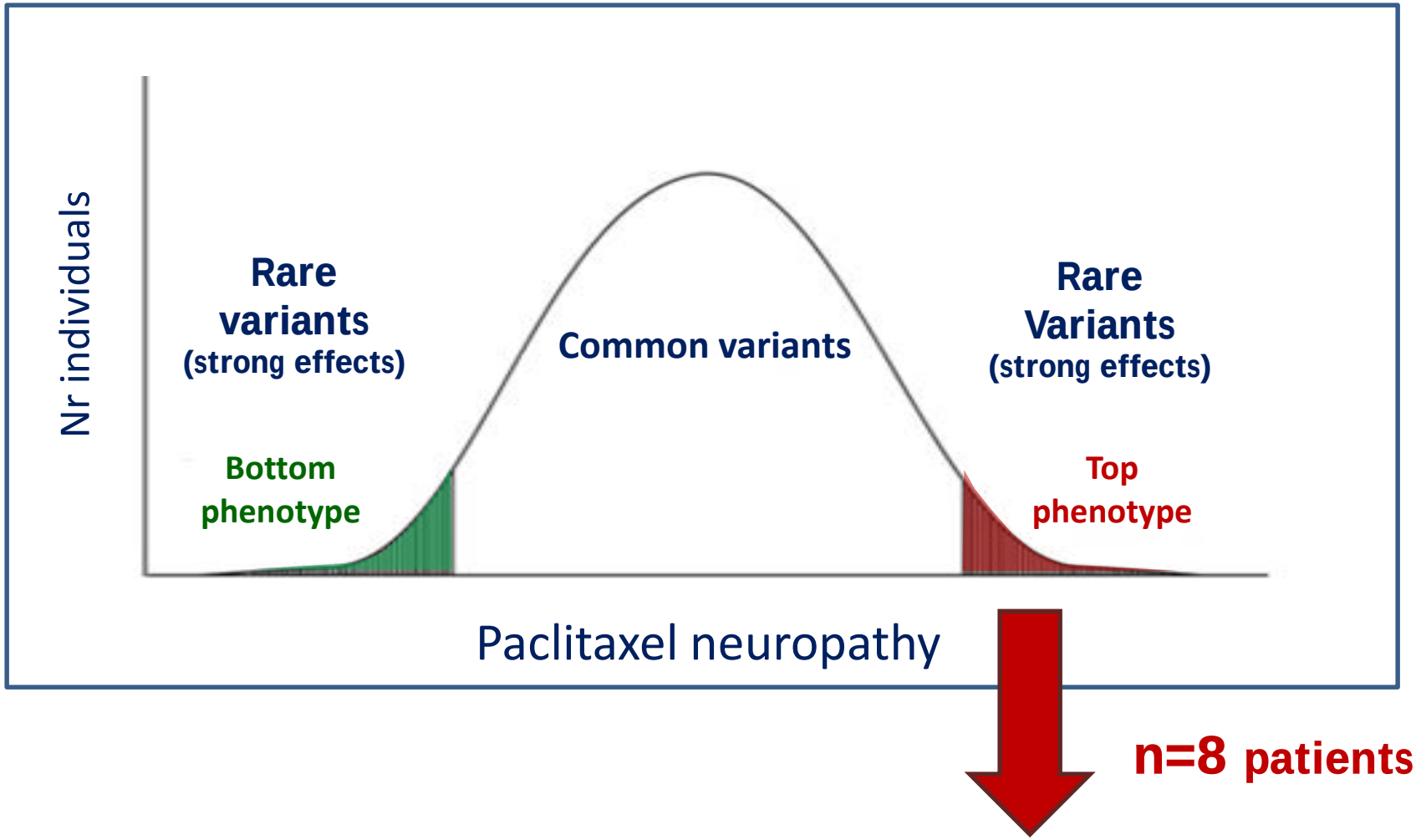

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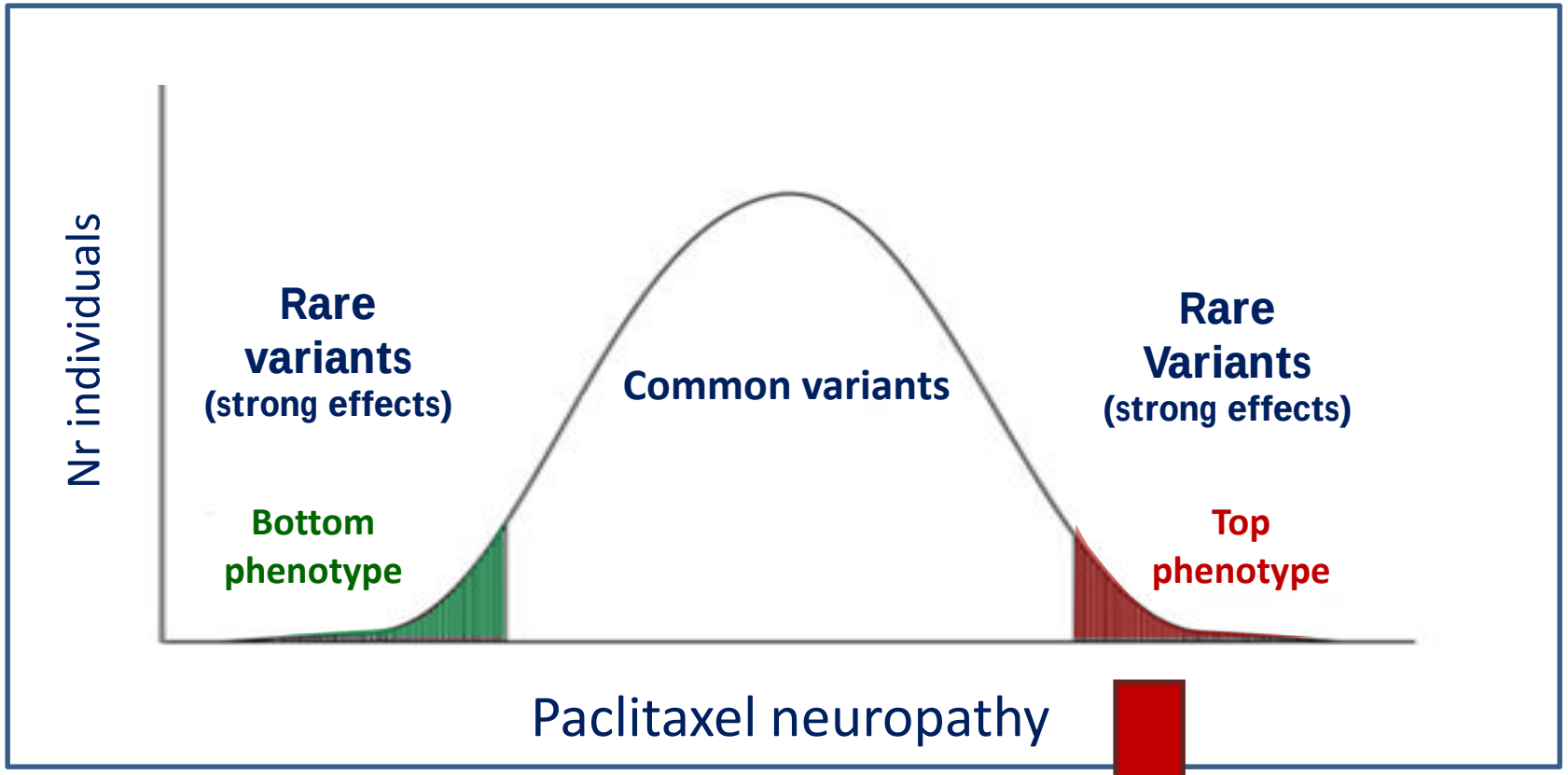

n=8 patients

Whole exome sequencing (WES)

Characteristics of the patients with extreme neuropathy

Characteristics	Clinical data
Age (y)	57 (39-79)
Breast cancer	8
First line paclitaxel treatment	8
Nr. Chemotherapy cycles	5-8
Paclitaxel dose at grade 3 neuropathy (mg/m^2)	750 (400-800)
Grade 3 neuropathy (CTC-NCI scale)	8
Treatment modifications due to neuropathy	8
Dose reduction	4
Treatment suspension	4
Duration of neuropathy grade 2-3 (years)	3.2 (1.6 – 5.5)

*Confounders ruled out (diabetes, alcohol intake, previous neuropathies..)

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Whole exome sequencing (WES)

- SureSelect XTHumanAllExon 50Mb
- HiSeq2000
- Median coverage 50x

DNA

Peripheral blood samples

(n=8)

SNVs and Indels

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

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SNVs and Indels

Variants in genes paclitaxel PD/ PK

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Coding variants in PD/PK genes

Gene	Variant / Name	Type of variant	Nr. variant carriers (het/hom)	MAF (%)		Effect prediction
				Obs	Eur	
TUBB2A	None	-	-	-	-	-
XKR4	None	-	-	-	-	-
EPHA5	N81T	Missense	2/0	12	7	Neutral
CYP3A4	P389S/ novel (*25)	Missense	1/0	6	ND	Defective
	P488Tfs*494/ *20	Frameshift	1/0	6	0.09	Defective
CYP2C8	R139K / *3	Missense	2/0	12	12	Neutral
	K399R / *3	Missense	2/0	12	12	Neutral
	I264M / *4	Missense	1/0	6	6	Neutral
	N21D	Missense	1/0	6	10	Neutral
ABCB1	N183S	Missense	1/0	6	0.2	Neutral
	A893S / 2677GT	Missense	5/1	43	43	Neutral
SLCO1B3	G653E	Missense	1/0	6	1	Neutral

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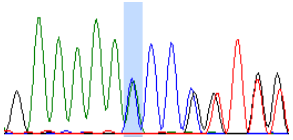
Rare *CYP3A4* high-impact variants in extreme neuropathy patients

Indel: Nonsense variant

P488Tfs*494 (*CYP3A4*20*)



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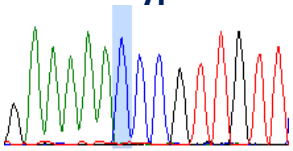

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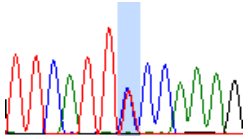
P389S



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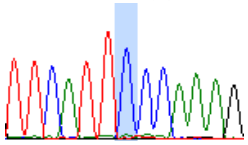

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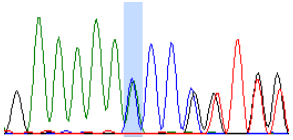

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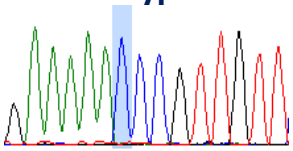

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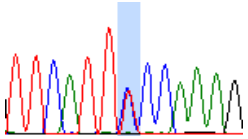

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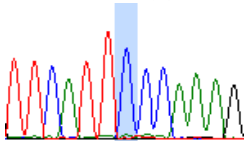

***CYP3A4* wt**

~GGACTTCTTCAACCAGAAAAACCCGTTGTTCTAAAGGTTGAGTCAAGGGATGGCACCGTAAGTGGAGCCTGA
G L L Q P E K P V V L K V E S R D G T V S G A *

***CYP3A4*20**

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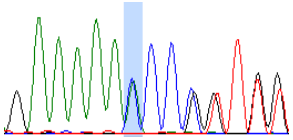

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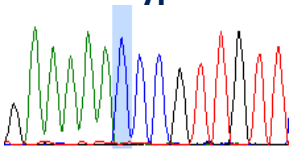

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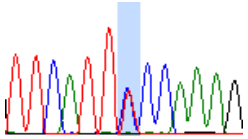

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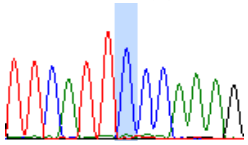

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  - Protein devoid of functional activity
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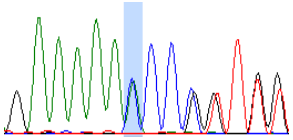

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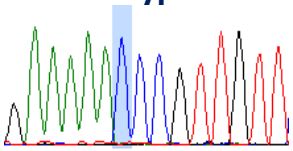

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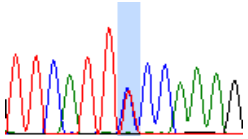

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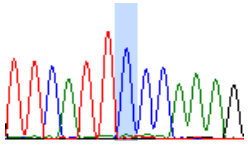

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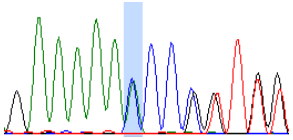

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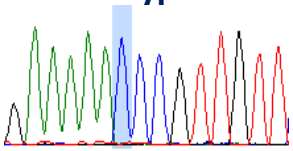

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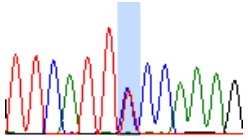

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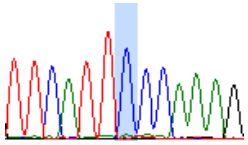
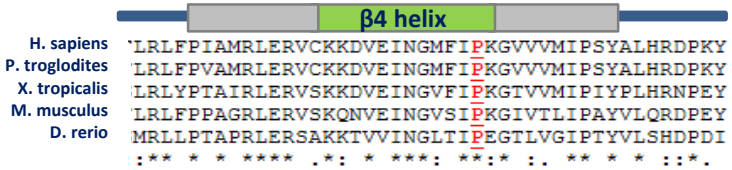


- Non-described
- Conserved β-helix



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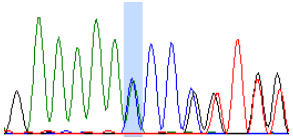

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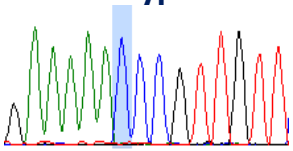

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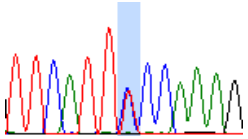

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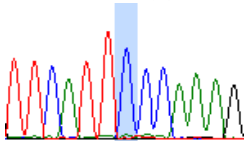

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- Non-described
- Conserved β-helix
- Defective (4/4 software prediction)

Screening for *CYP3A4* coding variants

N = 228 patients

-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***

Screening for *CYP3A4* coding variants

Summary of *CYP3A4* variants

Patient	*CYP3A4* variant effect	*CYP3A4* variant allele	Detection technique	Max. Neuropathy grade	Total nr. cycles	Treatment modifications	Time with neuropathy (months)
1	**Loss-of-function**	*20	**WES**	**3**	8	**Red (cycles 4 & 8)**	(35)
2		*20	DHPLC	**3**	8	**Red (cycle 4)**	(9)
3		*20	DHPLC	**3**	12	**Red (cycle 9)**	(6)
4		*20	DHPLC	**3**	11	No	16
5	**Decreased activity**	P389S (*25)	**WES**	**3**	6	**Susp (cycle 6)**	19
6		R130Q (*8)	DHPLC	1	12	No	6
7	**Pending**	L475V (*26)	DHPLC	**3**	8	No	(19)

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3		*20	DHPLC	**3**	12	**Red (cycle 9)**	(6)
4		*20	DHPLC	**3**	11	No	16
5	**Decreased activity**	P389S (*25)	**WES**	**3**	6	**Susp (cycle 6)**	19
6		R130Q (*8)	DHPLC	1	12	No	6
7	**Pending**	L475V (*26)	DHPLC	**3**	8	No	(19)

Screening for *CYP3A4* coding variants

Summary of *CYP3A4* variants

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Eiselt *et al*, *Pharmacogenetics* (2001)

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Functional characterization (*25 & *26)

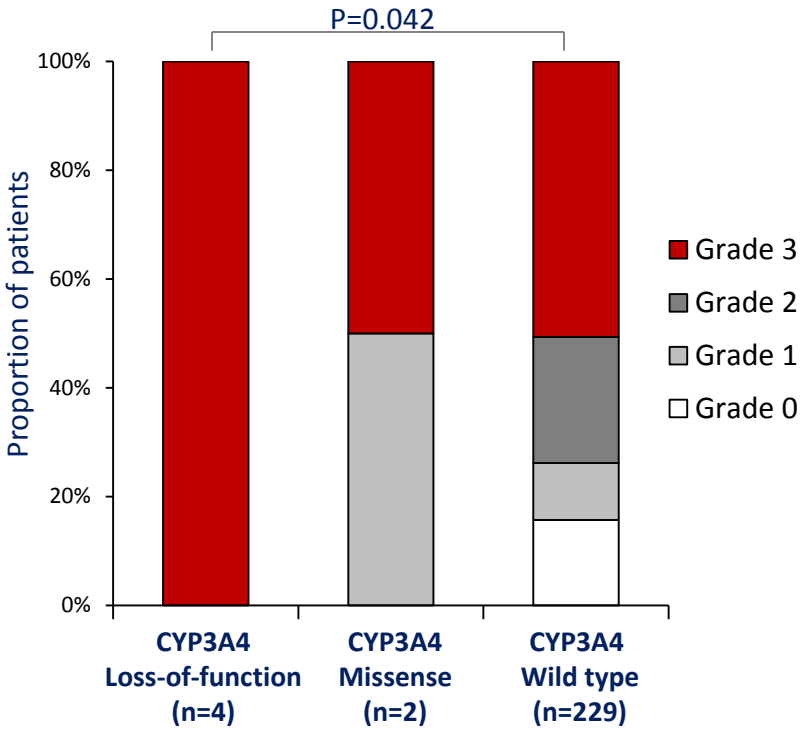
Mi-Young Lee
Magnus Ingelman-Sundberg

(Abstract **P-187**)

Karolinska Institutet

Are *CYP3A4* defective variants clinically relevant?

2-fold higher risk of Grade 3 neuropathy



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

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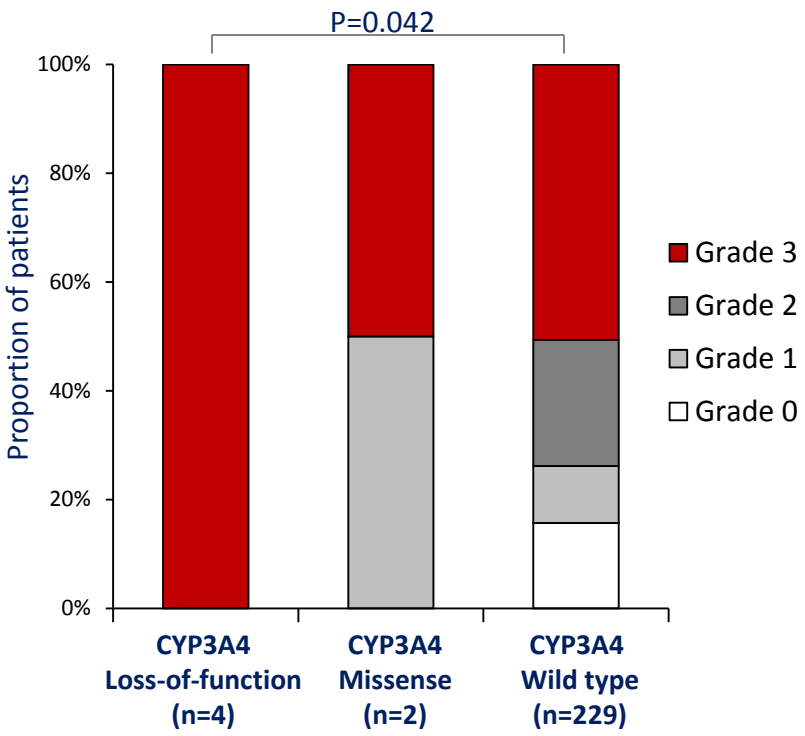
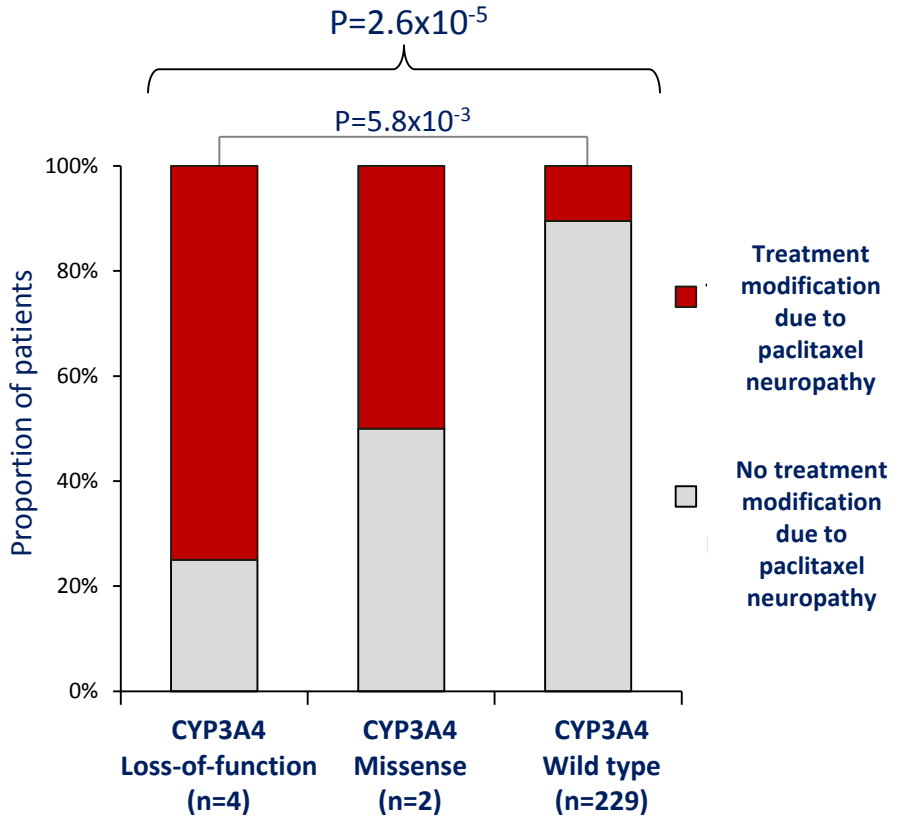


7- fold higher risk of treatment modifications



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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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- 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

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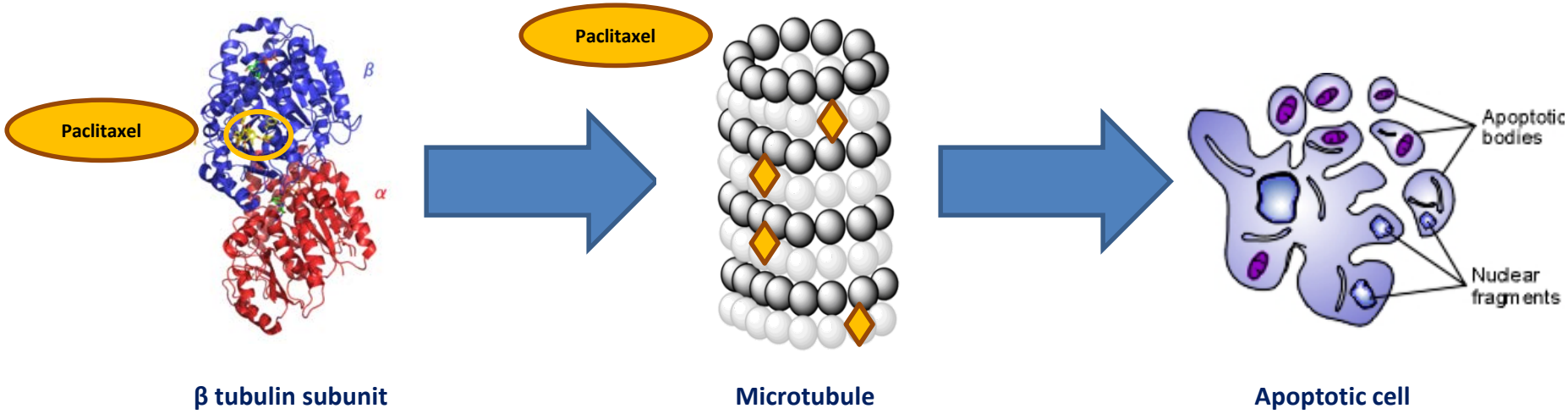
Founding

Gobierno de España – Ministerio de Economía y Competitividad

Obra Social Fundación "la Caixa"

Paclitaxel



- **Effective therapy** for many solid tumors (breast, ovarian, lung, pancreas)
- Dose limiting toxicity: **peripheral neuropathy**

Wani et al. *J Am Chem Soc.* (1971)
Schiff et al. *Nature* (1979)

Genetic markers of paclitaxel neuropathy

TUBB2A

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

GWAS metanalysis

-Balwin, *Clin Cancer Res* (2012)

-Leandro-Garcia, *J Med Genet* (2013)

EPHA5 → P=1.4 x 10^{-9}

XKR4 → P=3.1 x 10^{-8}

CYP2C8

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

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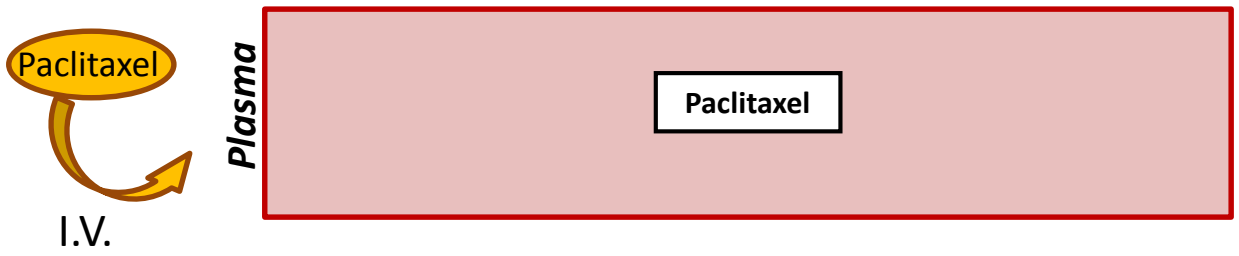

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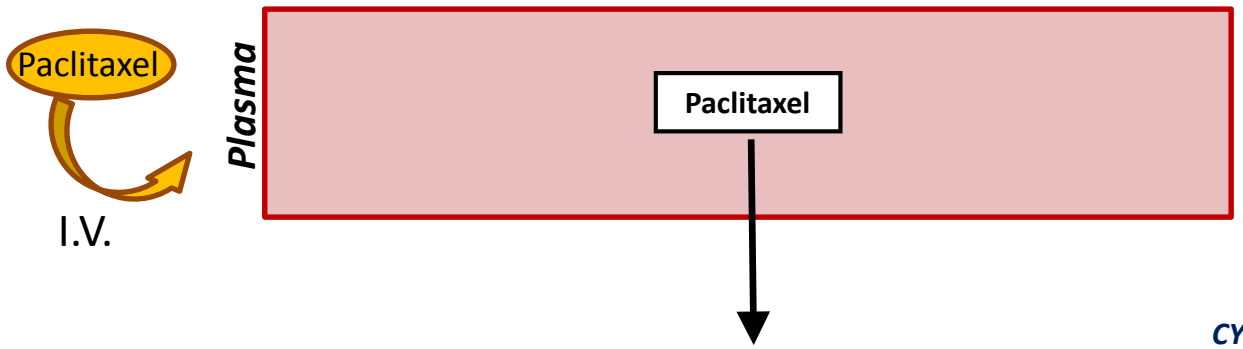

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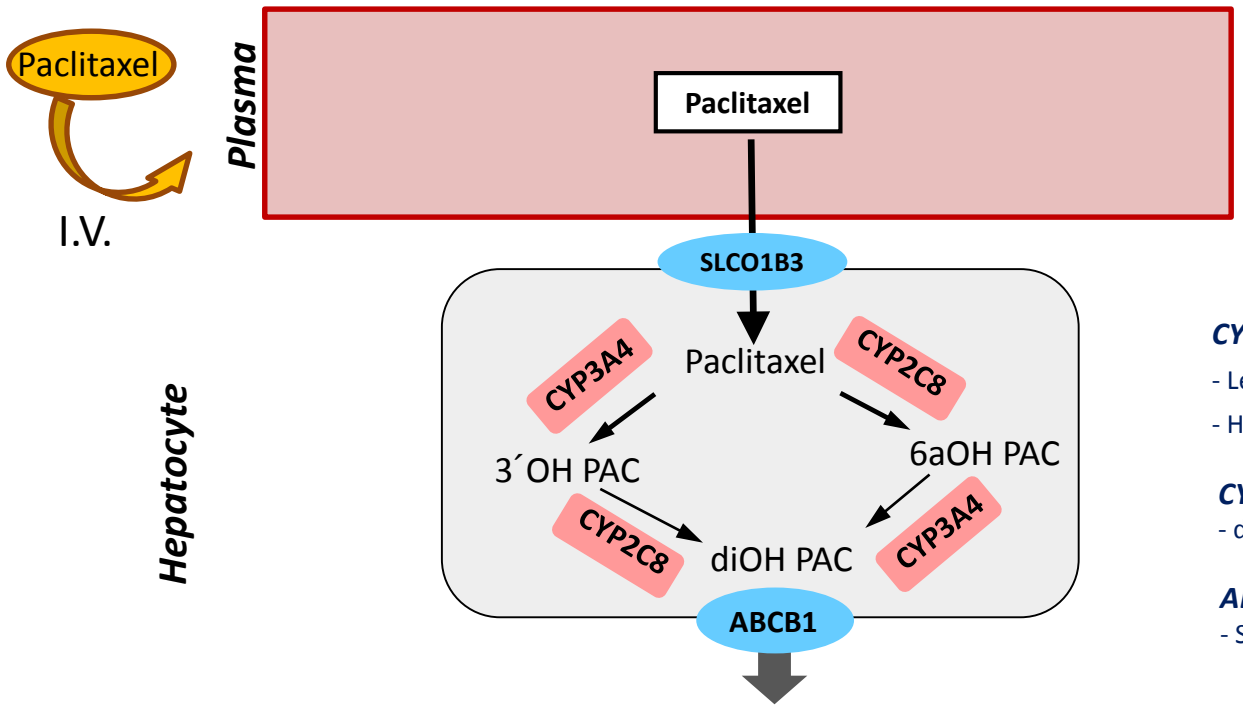

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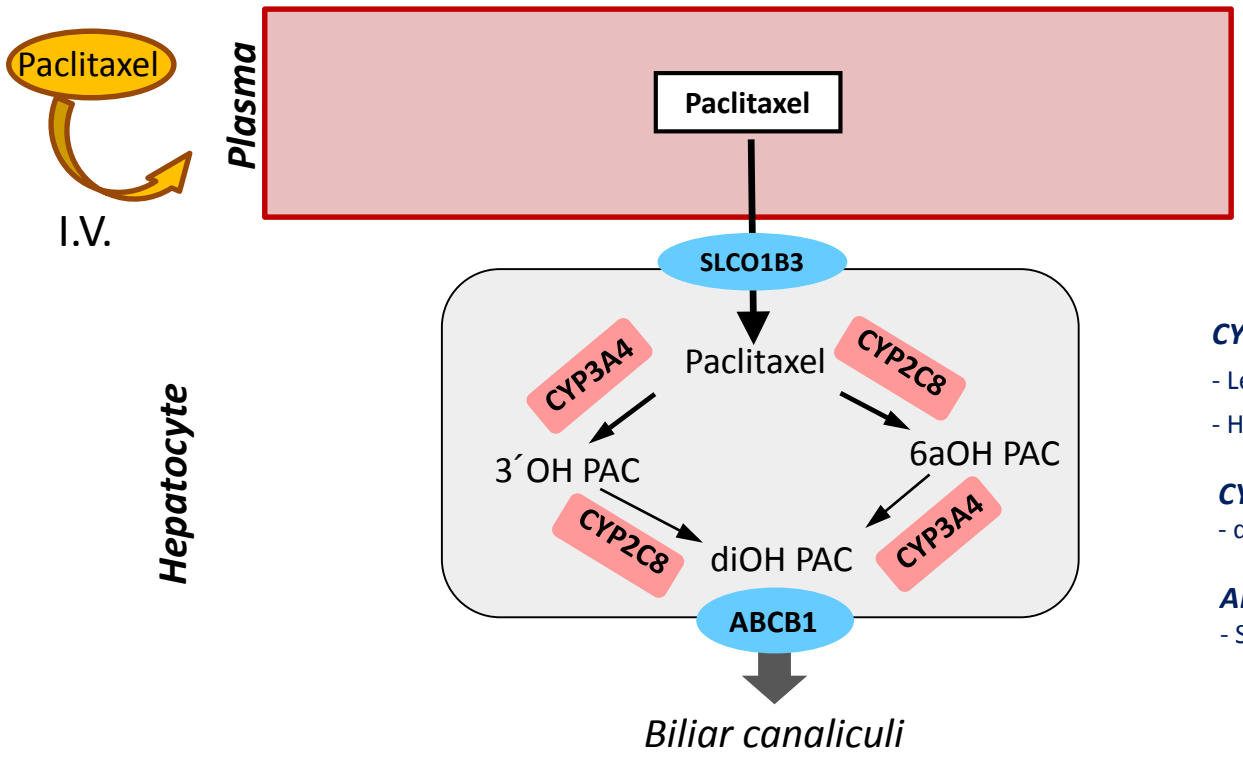

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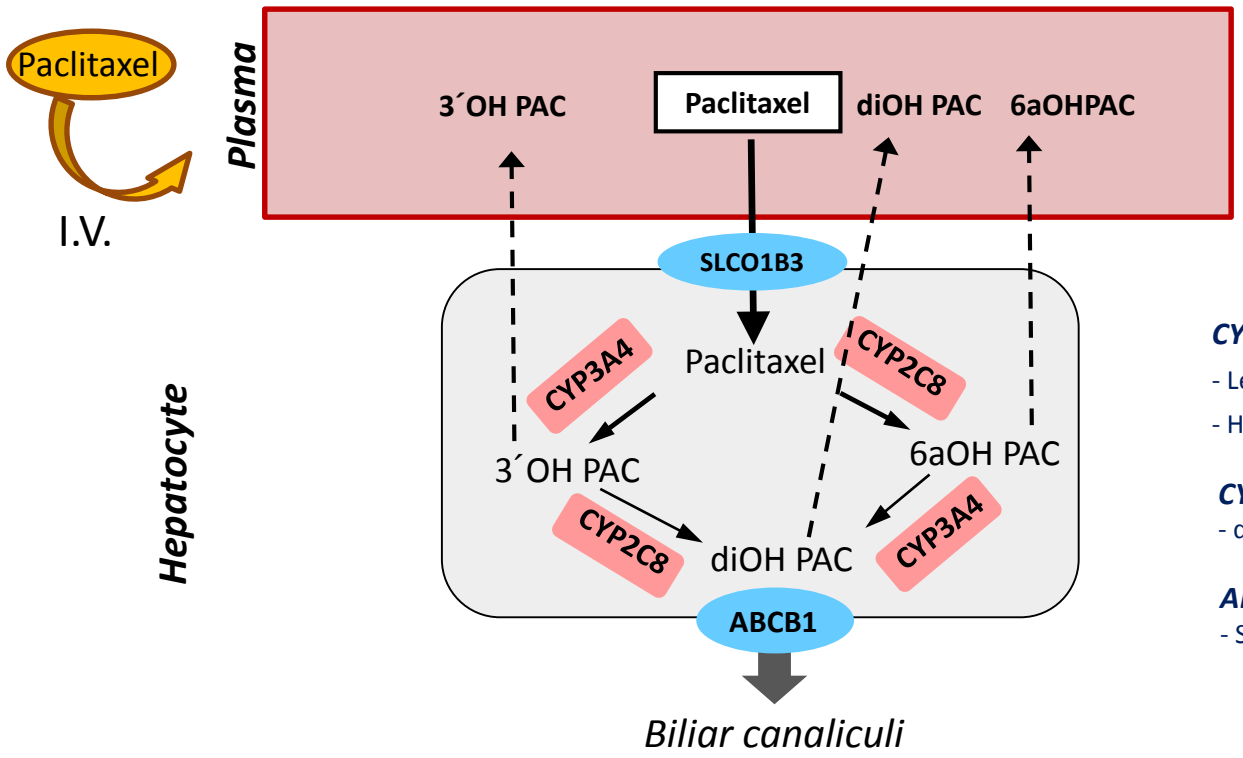

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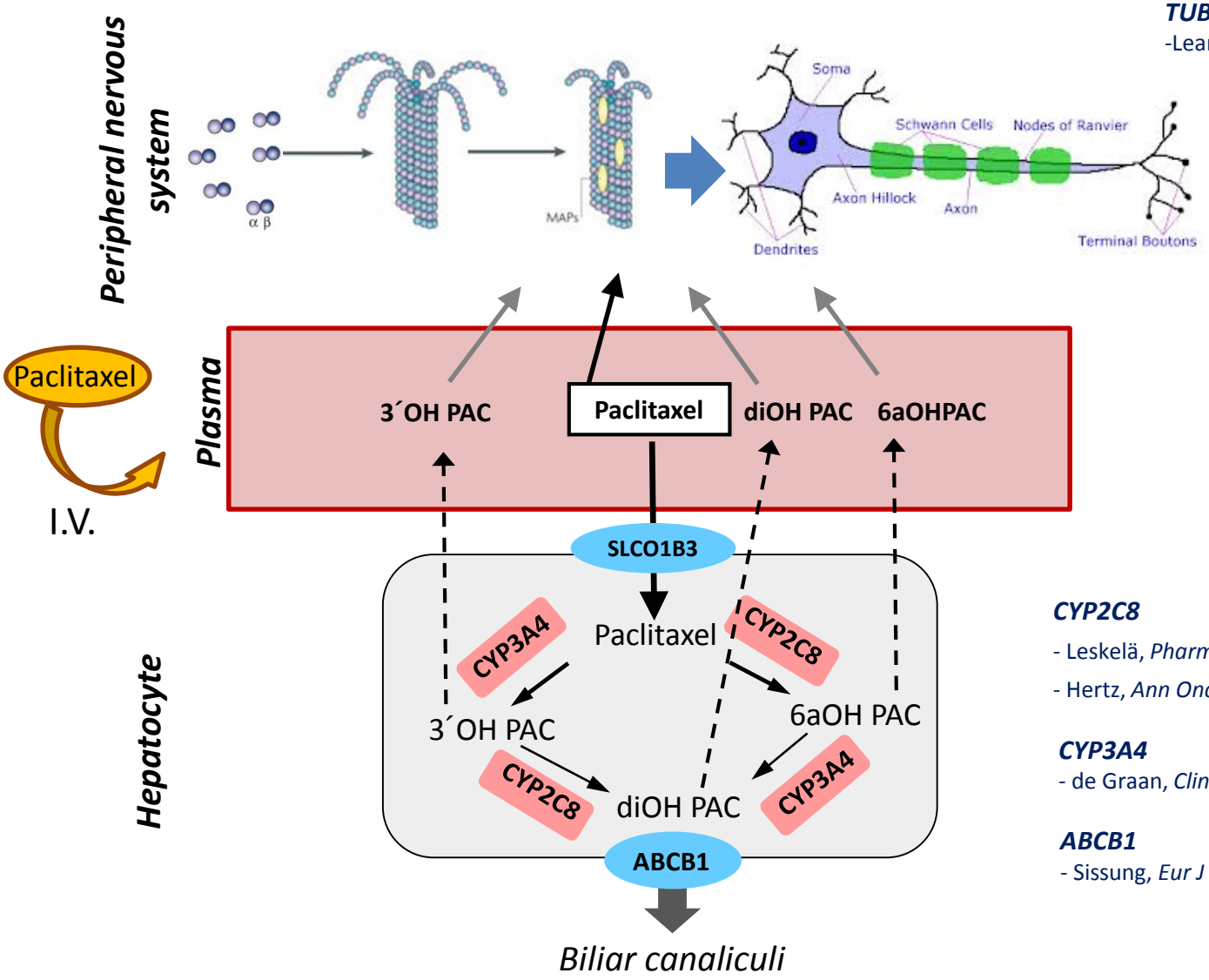

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Genetic markers of paclitaxel neuropathy: genome wide approaches (GWAS)

- Balwin *Clin Cancer Res* (2012)

  -Human 610k
  -Paclitaxel 175mg/m^2, every 14 d
  -Breast cancer, n=855
  -Accumulated Pac dose to G2 neuropathy

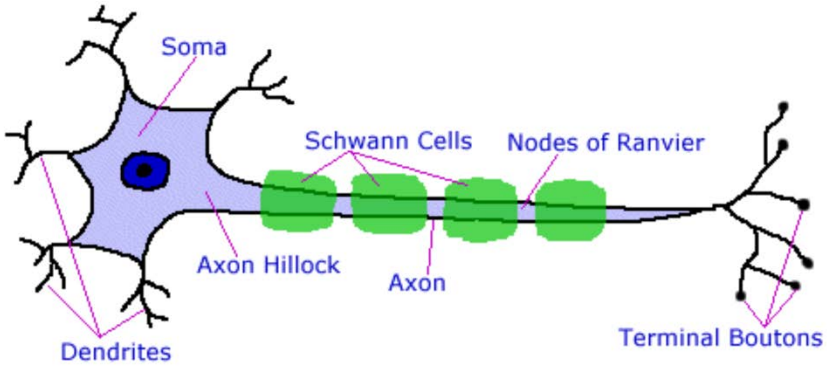
- Leandro-Garcia *J Med Genet* (2013)

  -Human 660WQuad
  -Paclitaxel 175mg/m^2 + 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 x 10^{-9}** (1x10^{-6}; 3x10^{-4}), MAF= 0.38

XKR4 (rs4737264): HR=1.7, **P=3.1 x 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)

Pharmacokinetics

CYP2C8

- Leskelä, *Pharmacogenomics J* (2011)
- Hertz, *Ann Oncol* (2013)

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

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

CYP3A4

- de Graan, *Clin Cancer Res* (2013)

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

ABCB1

- Sissung, *Eur J Cancer* (2006)

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

Pharmacodynamics

TUBB2A

- Leandro-Garcia, *Clin Cancer Res* (2012)

***TUBB2A* -101 &-112 variants**
PROTECTION P=0.021, **(MAF=0.29)**

Do patients with *CYP3A4* defective alleles have functional variants previously associated with neuropathy?

Gene	Variant name	Nr. of neuropathy risk alleles						
		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
CYP3A4	Frameshift (**20)*	**1**	**1**	**1**	**1**	0	0	0
	Novel P389S (**25*)	0	0	0	0	**1**	0	0
	Novel L475V (**27*)	0	0	0	0	0	**1**	0
	R130Q (**8*)	0	0	0	0	0	0	**1**
CYP3A4	*CYP3A4*22*	**1**	0	0	0	0	0	0
CYP2C8	*CYP2C8*3*	**1**	**1**	0	0	0	0	**1**
EPHA5	rs7349683	0	**2**	0	0	**1**	**2**	0
XKR4	rs4737264	**1**	**1**	0	0	**1**	0	**1**
***CYP3A4* coding variants**		**Loss-of-function (no activity)**				**Missense (decreased activity)**		
Paclitaxel-induced neuropathy (grade/ dose modif.) [a]		3/ Red (2)	3/ Red	3/ Red	3/ -	3/ Susp	3/ -	1/ -

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CYP2C8	*CYP2C8*3*	**1**	**1**	0	0	0	0	**1**
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XKR4	rs4737264	**1**	**1**	0	0	**1**	0	**1**
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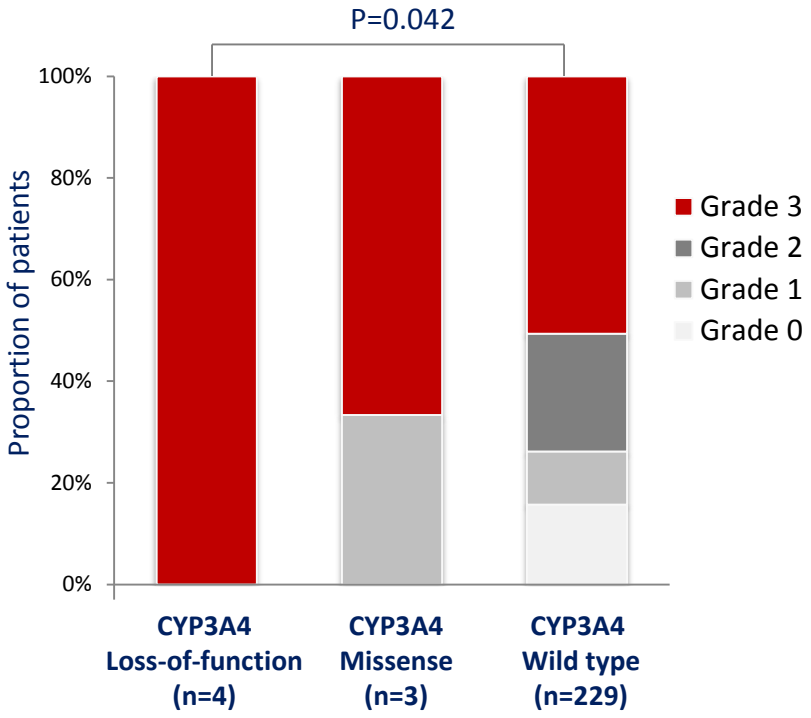

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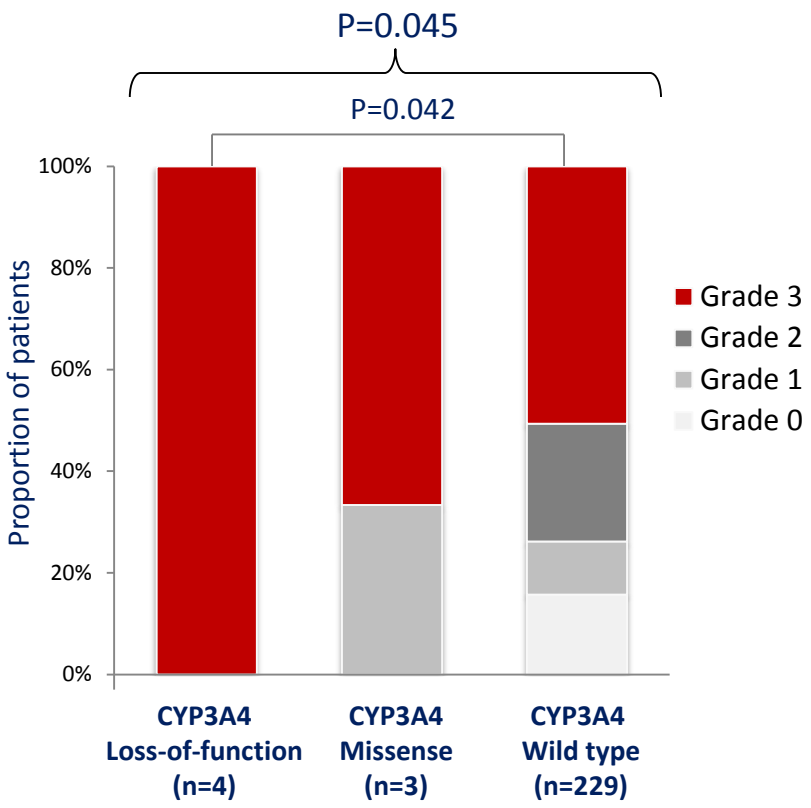
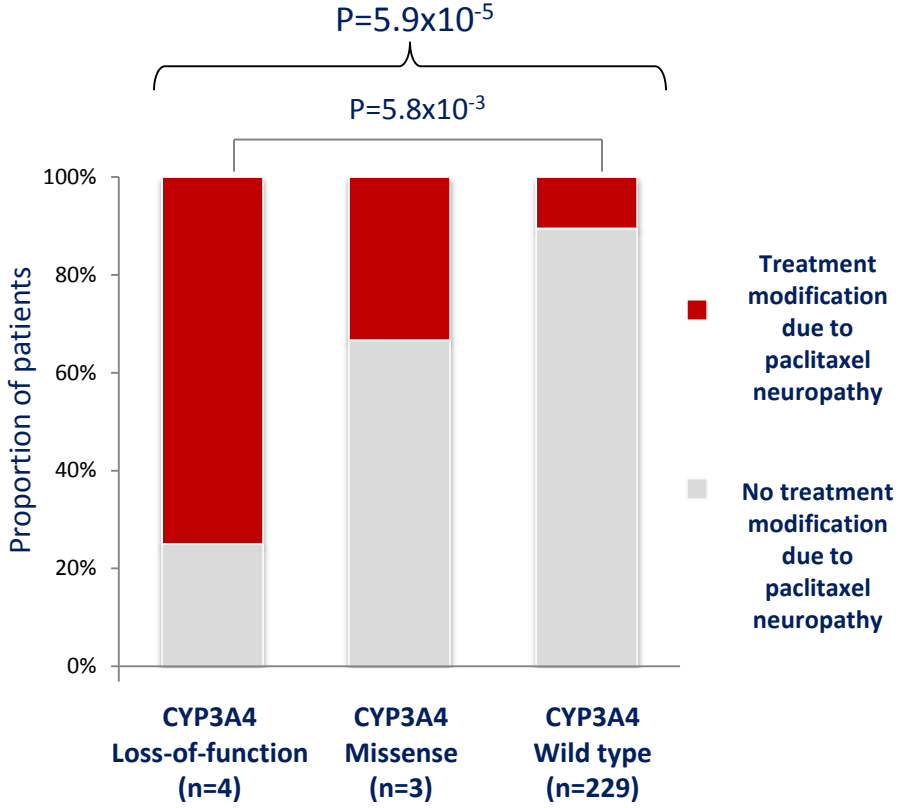


7- fold higher risk of treatment modifications



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