Title: Regulatory polymorphisms in β-tubulin IIa are associated with paclitaxel-induced peripheral neuropathy

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TRANSLATIONAL RELEVANCE

Paclitaxel is a microtubule binding drug widely used to treat several solid tumours, such as breast, ovary and lung. The current paclitaxel dose limiting toxicity is peripheral neuropathy, which is dose-cumulative and occurs in about 1/3 of patients. It exhibits a large inter-individual variability on unknown molecular basis, with some patients asymptomatic while others discontinue paclitaxel treatment due to the neuropathy, with severe cases with irreversible peripheral axonal damage. In this study we provide novel insights into the biology underlying paclitaxel neurotoxicity inter-individual variability by using different cell line models and an outstanding series of 214 well characterized patients treated with paclitaxel. We identified two common regulatory polymorphisms in the proximal promoter of β-tubulin IIa, the therapeutic target of paclitaxel, that confer an increased transcription rate and protect from paclitaxel-induced peripheral neuropathy (HR=0.62, 95% CI=0.42-0.93, P=0.021, multivariable analysis). These variants could provide the basis for an individualized paclitaxel pharmacotherapy.

ABSTRACT

Purpose: Peripheral neuropathy is the dose-limiting toxicity of paclitaxel, a chemotherapeutic drug widely used to treat several solid tumors such as breast, lung and ovary. Paclitaxel's cytotoxic effect is mediated through β-tubulin binding in the cellular microtubules. In this study we investigated the association between paclitaxel neurotoxicity risk and regulatory genetic variants in β-tubulin genes.

Experimental Design: We measured variation in gene expression of three β-tubulin isotypes (I, IVb and IIa) in lymphocytes from 100 healthy volunteers, sequenced the promoter region to identify polymorphisms putatively influencing gene expression and assessed the transcription rate of the identified variants using luciferase assays. To determine whether the identified regulatory polymorphisms were associated with paclitaxel neurotoxicity, we genotyped them in 214 patients treated with paclitaxel. In addition, paclitaxel-induced cytotoxicity in lymphoblastoid cell lines was compared to β-tubulin expression as measured by Affymetrix exon array.

Results: We found a 63-fold variation in β-tubulin IIa gene (*TUBB2A*) mRNA content and three polymorphisms located at -101, -112, and -157 in *TUBB2A* promoter correlated with increased mRNA levels. The -101 and -112 variants, in total linkage disequilibrium, conferred *TUBB2A* increased transcription rate. Furthermore, these variants protected from paclitaxel-induced peripheral neuropathy (HR=0.62, 95% CI=0.42-0.93, P=0.021, multivariable analysis). In addition, an inverse correlation between *TUBB2A* and paclitaxel-induced apoptosis (P=0.001) in lymphoblastoid cell lines further supported that higher *TUBB2A* gene expression conferred lower paclitaxel sensitivity.

Conclusions: This is the first study demonstrating that paclitaxel neuropathy risk is influenced by polymorphisms regulating the expression of a β-tubulin gene.

INTRODUCTION

Paclitaxel is a microtubule binding drug widely used for the treatment of several solid tumours, such as breast, ovary and lung (1). Paclitaxel binds the β-subunit of the tubulin dimers, the main components of cellular microtubules (2), leading to their stabilization, cell cycle block and cell death (3, 4). The current paclitaxel dose limiting toxicity is peripheral neuropathy (5, 6), which is predominantly sensory, and develops as a painful, debilitating and symmetrical distal axonal neuropathy (7, 8). Although the mechanisms causing this toxicity have not been precisely determined, it is clear that the microtubule mediated axonal transport is affected (9-11). Paclitaxel neurotoxicity is dose-cumulative, with some clinical factors influencing toxicity risk (12, 13). However, a large part of the inter-individual variability remains unexplained, and while some patients are asymptomatic others have to discontinue paclitaxel treatment due to the neuropathy. The symptoms usually disappear over months after paclitaxel treatment is stopped, but severe cases can have irreversible peripheral axonal damage. Our group and others have investigated the contribution of genetic variation in the paclitaxel pharmacokinetic pathway to neurotoxicity risk (14, 15), however, a large part of paclitaxel-induced neurotoxicity variability remains unexplained.

Although neuron β-tubulins are the therapeutic target that mediates paclitaxel neurotoxicity, these molecules have not been investigated in relation to the neuropathy. We have previously shown that neuronal microtubules are formed by six different isotypes: IVa, IIa, IVb, IIb, I and III, with β-tubulin IVa and IIa being the majority forms and constituting more than 75% of the total β-tubulin content in brain (16). This

tissue contains the highest amounts of β-tubulin, probably reflecting the importance of the extensive neuronal cytoskeleton for the diverse functions of the human neurons. β-tubulin I and IVb are ubiquitous isotypes, isotype IIa has a broad expression, while the expression of β-tubulin IIb, III, IVa is mainly restricted to neurons (16).

β-tubulins are highly conserved proteins, and polymorphisms leading to amino acid changes have been ruled out for all isotypes except for the hematologic-specific β-tubulin VI (17). In fact, missense variants in the neuron-specific β-tubulins IIb and III are pathogenic and lead to a spectrum of severe neuronal disorders (18, 19). Concerning variations in gene expression, β-tubulin III has been found over-expressed in tumors, and this event has been associated with poor prognosis and altered drug response in various tumour types (20-22). However, constitutive variability in the expression of these isotypes due to regulatory polymorphisms has not been investigated.

In this study we show that there is a large inter-individual variability in β-tubulin IIa mRNA expression and that two genetic variants in total linkage disequilibrium in the promoter region of the β-tubulin IIa gene (*TUBB2A*) are involved in this variation. Furthermore, genotyping of 214 patients treated with paclitaxel demonstrated that these polymorphisms are associated with paclitaxel neuropathy risk. In addition, an association between paclitaxel-induced apoptosis and β-tubulin IIa expression was further confirmed using cell lines.

MATERIALS AND METHODS

Human biological samples

Lymphocytes were isolated from total peripheral blood samples from 100 healthy volunteers by density gradient separation in Histopaque®-1077 (Sigma Aldrich, St. Louis, USA) as previously described (23). DNA from 214 cancer patients treated with paclitaxel were collected with the collaboration of one Spanish and two Swedish centers: 118 patients corresponded to the Hospital Universitario Fundación Alcorcón (15), 63 to the Karolinska Institutet, and 33 to the Linköping University (24). Ovary, lung and breast cancer were the most common malignancies from the patients, grade 3 neurotoxicity was observed in 11% of the patients and grade 2 in 39%. Patient characteristics, chemotherapy regimens and neurotoxicity data are summarized in Table 1. The collection of samples was approved by the corresponding Internal Ethical Review Committee and all patients signed a written informed consent before the collection of a blood or saliva sample.

RNA isolation and quantitative RT-PCR

RNA was extracted from lymphocytes using TRI-reagent (Molecular Research Center Inc., Cincinnati, USA) and the concentration quantified by using Nanodrop ND-1000 (Wilmington, DE, USA). One µg of total RNA was reverse transcribed using Superscript II (Invitrogen, Carlsbad, USA) and an oligo dT14 primer following the manufacturer's instructions. The mRNA content of the different β-tubulin isotypes was quantified by qRT-PCR with the Sequence Detection System 7900HT (Applied Biosystems Foster City, USA) using conditions, primers and probes previously described (16) (Supplementary Table 1). Normalization was carried out with the internal standard beta-glucuronidase (*GUS*). Negative controls were included in all PCR series

and assays were carried out in triplicates. The delta-delta Ct method was used for the calculation of mRNA content (25).

DNA isolation, sequencing and genotyping

Genomic DNA from lymphocytes was isolated using the FlexiGene DNA Kit (QIAGEN, Valencia, USA). DNA concentration was determined using PicoGreen dsDNA quantification reagent (Invitrogen). For sequencing, *TUBB2A* promoter region was amplified by PCR using specific primers (Supplementary Table 1). PCR amplification products were purified using the PCR Purification Kit (QIAGEN) and run on an ABI PRISM 3700 DNA Analyzer capillary sequencer (Applied Biosystems). Genotyping for the *TUBB2A* polymorphism located at -112 A>G (rs909965) and -157 A>G (rs9501929) was performed in duplicates with the KASPar SNP Genotyping System (Kbiosciences, Herts, UK) using 15 ng of genomic DNA. All assays included DNA samples with known genotypes and negative controls. The sequence Detection System 7900HT (Applied Biosystems) was used for fluorescence detection and allele assignment.

***TUBB2A* promoter cloning, transient transfection and luciferase assay**

We amplified the promoter region of β-tubulin isotype IIa gene (-389 to -15, nucleotide positions referring to *TUBB2A* translation start site ATG, +1) using specific primers that introduced *Xho*I and *Hind*III cleavage sites (Supplementary Table 1). The PCR product was cloned into the promoterless pGL3-Basic *Firefly* luciferase reporter vector (Promega) to generate pGL3B_WT plasmid. Mutagenesis was performed by means of the DNA EXPRESS INC (Montreal, Canada) to generate a plasmid with -101C (rs909964) and -112G (rs909965) nucleotide changes in the promoter region of *TUBB2A* (pGL3B_-101C/-112G) and another plasmid with -157G (rs9501929)

nucleotide change (pGL3B_-157G). The sequence of all the constructs was verified by DNA sequencing.

H1299 cells, derived from non-small cell lung cancer, were plated in 24-well plates and were transiently transfected with 0.3µg of pGL3-Basic vector (EV) or the appropriate reporter constructs (pGL3B_WT, pGL3B_-101C/-112G, and pGL3B_-157G), and the internal reference Renilla plasmid pRL-SV40 (Promega), using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were harvested 48 hours after transfection and lysates were used to measure *Firefly* and *Renilla* luciferase activities using the Dual Luciferase Reporter Assay System (Promega) in a Synergy™ 4 Hybrid Microplate Reader (Biotek). Three independent experiments were performed using triplicates.

Paclitaxel induced apoptosis in lymphoblastoid cell lines

HapMap lymphoblastoid cell lines from a population with Northern and Western European ancestry from Utah, USA (HAPMAPPT01, CEU, n = 77) were treated with 12.5 nM paclitaxel and apoptosis (caspase-3 and -7 activity) was measured 24 h after drug treatment using the Caspase-Glo 3/7 Assay (Promega, Madison, WI) (26). Gene expression data for *TUBB2A* in this population came from a previously published Affymetrix exon microarray analysis (27). A general linear model was constructed to test for association between $\log_2$-transformed *TUBB2A* expression and $\log_2$-transformed paclitaxel-induced caspase activity. A Toeplitz covariance structure with two diagonal bands was used to allow for familial dependencies in the data as described previously (28).

Statistical analysis

Statistical analyses were carried out using SPSS software package version 17.0 (SPSS, Chicago, IL, USA). The method of Kolmogorov-Smirnov was used to test for

normality. The Mann-Whitney non-parametric statistical test was applied to compare median β-tubulin IIa mRNA expression content. Associations between genotypes and paclitaxel neurotoxicity risk were tested using Cox regression analysis, modeling the cumulative dose of paclitaxel up to the development of grade 2 neurotoxicity. Patients with no or minimal adverse reaction (grade 0/1) were censored at total cumulative dose. Multivariable analysis was performed including relevant clinical factors as covariates. Paired t-test was used to compare the normalized luciferase activity (*Firefly*/ *Renilla*) of the different constructs. Differences were considered significant when P-values were less than 0.05.

RESULTS

β-tubulin IIa shows large inter-individual differences in expression related to polymorphisms in the promoter region

We previously showed that six β-tubulin isotypes (IVa, IIa, IVb, IIb, I and III) are expressed in neurons (16). Among these isotypes, IIa, IVb and I, are expressed in a wide number of tissues, including peripheral blood leukocytes, where their mRNA expression can be easily and accurately measured through qRT-PCR. Thus, we quantified the expression of these three isotypes in leukocytes from 100 healthy volunteers. We found that β-tubulin IIa mRNA content was subjected to a large inter-individual variability, 63-fold variation in expression (Fig. 1A) while β-tubulins IVb and I showed a 2.5 and 2.2-fold variation in mRNA content, respectively (data not shown). Variation in β-tubulin IIa expression was also found at protein level, in concordance with mRNA contents (Suppl. Fig. 1).

To investigate whether this inter-individual variability in β-tubulin IIa mRNA expression could be due to genetic variability in the promoter region of *TUBB2A* gene, we sequenced the proximal promoter of the gene (300 bp) in individuals with high and low expression levels (>10000 and <2500 *TUBB2A* mRNA (r.u.) in n=9 and n=11 samples, respectively, Supplementary Table 2). Taking into account the differences between high and low *TUBB2A* expression groups, and the linkage disequilibrium between variants, we selected -101T>C, -112A>G, and -157A>G variants (corresponding to rs909964, rs909965 and rs9501929, respectively) as potentially associated with higher *TUBB2A* expression. The minor allele frequencies of these polymorphisms in Caucasian population are 0.28, 0.28 and 0.05, respectively (http://www.1000genomes.org/). We found total linkage disequilibrium between -101T>C and -112A>G polymorphisms, while -157A>G was independent from the other

two (r^2<0.001) and in high linkage disequilibrium with -91 G>A (rs13219681) (r^2=0.72).

To elucidate whether these polymorphisms could be affecting β-tubulin IIa mRNA expression levels, we genotyped -112A>G and -157A>G in the 100 peripheral blood lymphocytes previously used to measure mRNA expression (Fig. 1B). Lymphocytes carrying the -157G variant showed a significantly higher *TUBB2A* mRNA content (P=0.02). All the remaining β-tubulin IIa high expressers were carrying the -101T/-112G variants, although the differences did not reach statistically significance. Lymphocytes simultaneously carrying -157G and -101C/-112G variants, showed a significantly higher expression than the wild type group (P=0.02).

TUBB2A -101C/-112G promoter variants show an increased transcription rate in luciferase assays

To determine whether the identified *TUBB2A* promoter variants had an effect on transcription rate, we determined the transcriptional capacity of the variant promoters by transfecting the pGL3 Basic vector, pGL3B_WT, pGL3B_-101C/-112G, and pGL3B_-157G plasmids into H1299 cells. The transcriptional activity of the promoter variants measured by luciferase assay was significantly higher for the -101C/-112G variant promoter than wild-type and -157G variant promoters (P=0.011 and P=0.018, respectively) (Fig. 2). No differences in transcriptional activity were found between -157G and wild type promoter.

Paclitaxel neurotoxicity risk is decreased in -101C/-112G carrier patients

Cancer patients treated with paclitaxel were genotyped for *TUBB2A* -101C/-112G and -157G polymorphisms and the genotypes were compared to the peripheral

neuropathy developed by the patients. As shown in Figure 3, we found that patients carrying the -101C/-112G variants had a significantly decreased risk of developing paclitaxel neurotoxicity, with an estimated hazard ratio (HR) of 0.60 (95% CI=0.41-0.90, P=0.012). We confirmed that treatment schedule was an important covariate, with 80-90 mg/m^2 weekly scheme being more neurotoxic than 150-175 mg/m^2 every 21 days (HR=1.91, 95% CI=1.22-3.00, P=0.005) (29), thus, we included paclitaxel schedule as a covariate in a multivariable analysis. *TUBB2A* -101C/-112G variants showed a similar association with neuropathy protection in a Cox regression analysis adjusting for treatment schedule (HR=0.62, 95% CI=0.42-0.93, P=0.021). When we analysed *TUBB2A* -157G variant we did not find statistically significant differences in paclitaxel neurotoxicity in the patients.

Increased *TUBB2A* expression is associated with decreased paclitaxel-induced apoptosis

Previously, we evaluated paclitaxel-induced apoptosis as measured by caspase 3/7 activation in 77 CEU lymphoblastoid cell lines from the International HapMap Project (30). *TUBB2A* expression was determined in the same LCLs using Affymetrix exon expression array as described previously (27). To determine whether *TUBB2A* expression and paclitaxel cytotoxic activity could be related, we compared the expression of this gene with paclitaxel-induced apoptosis. A statistically significant inverse correlation between *TUBB2A* gene expression measured and paclitaxel-induced apoptosis was found (P=0.001, Fig. 4). This indicates higher *TUBB2A* gene expression confers resistance to paclitaxel-induced apoptosis.

DISCUSSION

In this work we found a large inter-individual variability in the expression of β-tubulin IIa. This isotype forms part of the neuronal microtubules, which are the therapeutic target of paclitaxel in neurons. Thus, we hypothesized that variation in β-tubulin IIa expression could be explained by regulatory polymorphisms in the promoter region of this gene and that these could contribute to the differences in toxicity observed in patients treated with paclitaxel. Specifically, two polymorphisms in linkage disequilibrium, -101T>C and -112A>G, showed an increased transcription rate in luciferase functional assays. Furthermore, patients carrying *TUBB2A* -101C/-112G promoter variants, had a significantly reduced risk of developing neuropathy during paclitaxel treatment. The correlation between higher *TUBB2A* gene expression and lower paclitaxel sensitivity in cell line models provides biological evidence that supports this association.

Previous studies suggest that genetic variation could contribute to paclitaxel neurotoxicity risk. In this respect, paclitaxel cytotoxicity heritability is higher than 0.50 and amongst the highest from a range of the cytotoxic drugs tested in lymphoblastoid cell lines (31). Among the genes that have previously been associated with paclitaxel neurotoxicity risk, most are involved in paclitaxel clearance pathway, *CYP2C8*, *CYP3A5* and *ABCB1* (14, 15, 32). Genes involved in other pathways have also been suggested to influence paclitaxel neurotoxicity. In this respect, two haplotypes of *FANCD2*, a DNA repair gene, were associated with the expression of this gene and increased paclitaxel neurological toxicity (33), suggesting an altered activity to repair chemotherapy-induced DNA damage. However, the precise mechanism by which this enzyme interferes with paclitaxel-induced neuropathy remains to be elucidated, since

paclitaxel does not produce DNA-breaks, but a potential role for DNA damage following mitotic arrest has been proposed for this drug.

This study constitutes the first evidence supporting that polymorphisms in the therapeutic target of paclitaxel, β-tubulin, can influence the clinical outcome of patients treated with this drug. Changes in β-tubulin isotype composition have been associated with paclitaxel tumor response (20-22). Specifically, increased tumor expression of β-tubulin II has been strongly associated with poor outcome in head and neck carcinoma patients treated with an induction chemotherapy that contains docetaxel, a paclitaxel analog (34). Furthermore, *TUBB2A* increased expression has been correlated with decreased drug sensitivity in paclitaxel resistant cell lines (35). These evidences are in agreement with our study, where we find a very significant correlation between high *TUBB2A* gene expression and lower paclitaxel-induced apoptosis in lymphoblastoid cell lines (P=0.001, Fig. 4). However, it is important to note that variation in additional genes likely accounts for additional inter-individual variability in caspase 3/7 activity (r^2=0.131; Fig. 4) In a similar way to the cell lines, we found that patients carrying *TUBB2A* polymorphisms leading to increased transcription rate had a decreased risk of developing paclitaxel neurotoxicity (HR=0.62, 95% CI=0.42-0.93, P=0.021; Fig. 3). All this data suggests that high amounts of β-tubulin II confers resistance to the action of taxanes. In this regard, the complex expression patterns of the multiple β-tubulin isotypes together with *in vitro* experiments, suggest a different functionality and drug sensitivity of the different isotypes (36-38), which could explain higher paclitaxel resistance with increased *TUBB2A* expression.

The great inter-individual variability that we found in *TUBB2A* expression reflects the high genetic variability that we found in *TUBB2A* promoter region (Supplementary table 2). Luciferase activity assays demonstrated that -101C/-112G variants were

functional and influenced transcription rate. The close proximity of -101/ -112 polymorphisms to the TATA box in *TUBB2A* core promoter, together with *in silico* predictions suggesting that several transcription factors binding sites could be affected by these polymorphisms (Suppl. Fig 2), further supports the functionality of these variants. Although *TUBB2A* -157G polymorphism was associated with increased *TUBB2A* mRNA content in lymphocytes, it did not affect luciferase activity and we did not find an association between this SNP and the patients' neurotoxicity risk, suggesting that this variant does not influence paclitaxel effects. However, the allele frequency of this polymorphism is relatively low (0.047) reducing the statistical power, and this variant may just be a marker in linkage disequilibrium with a regulatory variant located in another region of *TUBB2A* promoter. In addition, we cannot rule out that other *TUBB2A* promoter SNPs could also be contributing to the observed variability in expression and paclitaxel toxicity risk. Similarly, polymorphisms leading to a variable expression of other neuronal β-tubulins could also influence paclitaxel neurotoxicity. In this respect, we have ruled out variability in β-tubulin I and IVb expression, however, because IVa, IIb and III are mainly neuron-specific, we could not include them in our study.

In conclusion, in this study we found a large inter-individual variability in *TUBB2A* expression related to the higher transcriptional rate of the variant -101C/ -112G *TUBB2A* promoter. Furthermore, cell line models demonstrated that increased *TUBB2A* expression correlated with resistance to paclitaxel and in patients we found that -101C/ -112G *TUBB2A* regulatory polymorphisms conferred a significantly lower paclitaxel-induced neuropathy risk. This is the first study demonstrating an association between paclitaxel toxicity and regulatory polymorphisms in a therapeutic target of this drug (β-tubulin IIa). If confirmed in independent series, these polymorphisms could be

used as markers of paclitaxel-induced peripheral neurotoxicity risk, providing the basis for an individualized paclitaxel pharmacotherapy.

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FIGURE LEGENDS

Figure 1. Inter-indiviual variability in *TUBB2A* expression. A) *TUBB2A* mRNA content was measured by quantitative RT-PCR in 100 peripheral blood leukocytes from healthy donors, as described in Materials and Methods section. The horizontal bar represents the median value. **B)** The healthy donors were genotyped and grouped according to the polymorphisms located at -101, -112 and -157 in *TUBB2A* promoter region (rs909964, rs909965 and rs9501929). For each genetic group, β-tubulin IIa mRNA content is represented in a box plot. The boxes show the interquartile range, the horizontal line represents the median value for each group, and the whiskers extend to the minimum and maximum values. All nucleotide positions refer to *TUBB2A* translation start site (ATG, +1).

Figure 2. Effect of *TUBB2A* promoter variants on transcriptional activity. H1299 cells were transfected with pGL3-Basic (EV) and luciferase reporter plasmids with different polymorphisms in *TUBB2A* gene: pGL3B_WT (WT), pGL3B_-157G (-157G), and pGL3B_-101C/-112G (-101C/-112G). The cells were cotransfected with the pRL-SV40 plasmid containing the *Renilla* luciferase gene, which served as internal control of transfection efficiency. Promoter activities were calculated as the *Firefly*/*Renilla* signal ratios. Mean values with standard deviations for the entire data set (three transfections, each with three replicates) are shown. Paired t-test was used to test differences between the luciferase activities (P-values are shown).

Figure 3. Kaplan-Meier analysis of cumulative dose of paclitaxel up to the development of grade 2 neurotoxicity, according to -101C/-112G variants in

***TUBB2A*.** Patients treated with paclitaxel were grouped according to *TUBB2A* genotype. Those carrying one or two variant alleles had a significantly lower risk of paclitaxel induced neurotoxicity. The P value shown corresponds to univariable log-rank test.

Figure 4. Inverse correlation between *TUBB2A* gene expression and paclitaxel induced apoptosis. Lymphoblastic cell lines (CEU, n=77) were treated with paclitaxel to measure caspase 3/7 activation (apoptosis) and Affymetrix exon array was used to measure *TUBB2A* expression in the same cell lines, as described in Materials and Methods section. The graph shows a linear model comparing log_2-transformed *TUBB2A* expression and log2-transformed paclitaxel-induced caspase activity.

SUPPLEMENTRAY FIGURE LEGENDS

Supplementary Figure 1. β-tubulin II protein expression in peripheral blood leukocytes. β-tubulin II protein was detected in peripheral blood leukocytes from healthy volunteers with low and high *TUBB2A* mRNA expression by western blot using an antibody specific for IIa and IIb isotypes (clone 7B9; Covance, Emeryville, CA, USA). β-actin was used as control in the western blot.

Supplementary Figure 2. Putative binding sites for transcription factors in *TUBB2A* proximal promoter. Schematic representation of putative transcription binding sites that could be altered by the -101 and -112 *TUBB2A* variants (MATCH™ public version 1.0, using Matrix Search for Transcription Factor Binding Sites; http://www.gene-regulation.com/cgi-bin/pub/programs/match/bin/match.cgi, using as

core match and matrix match cut-offs 0.875 and 0.700, respectively). **A.** Wild type *TUBB2A* promoter. **B.** -101 and -112 variant *TUBB2A* promoter. The TATA box is in bold; polymorphisms at -101 and -112 positions are marked with arrows. Transcription factors affected by -101 and -112 polymorphism are shown in red and blue, respectively. Nucleotide positions refer to the translation start site (ATG, +1).

Figure 1

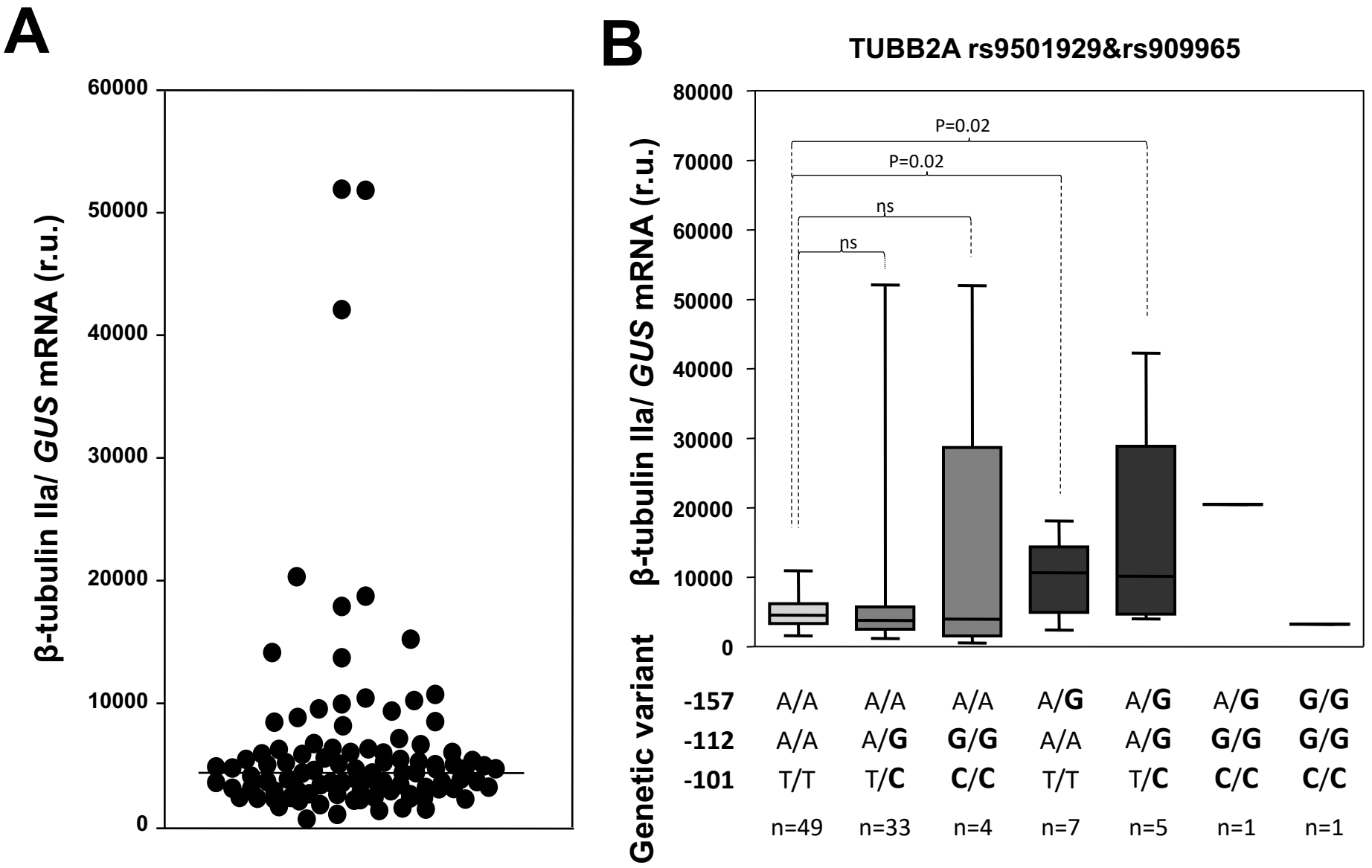
A
β-tubulin IIa/ GUS mRNA (r.u.)
60000
50000
40000
30000
20000
10000
0
B
TUBB2A rs9501929&rs909965
β-tubulin IIa/ GUS mRNA (r.u.)
80000
70000
60000
50000
40000
30000
20000
10000
0
P=0.02
P=0.02
ns
ns
Genetic variant
-157 A/A A/A A/A A/G A/G A/G G/G
-112 A/A A/G G/G A/A A/G G/G G/G
-101 T/T T/C C/C T/T T/C C/C C/C
n=49 n=33 n=4 n=7 n=5 n=1 n=1

Figure 2

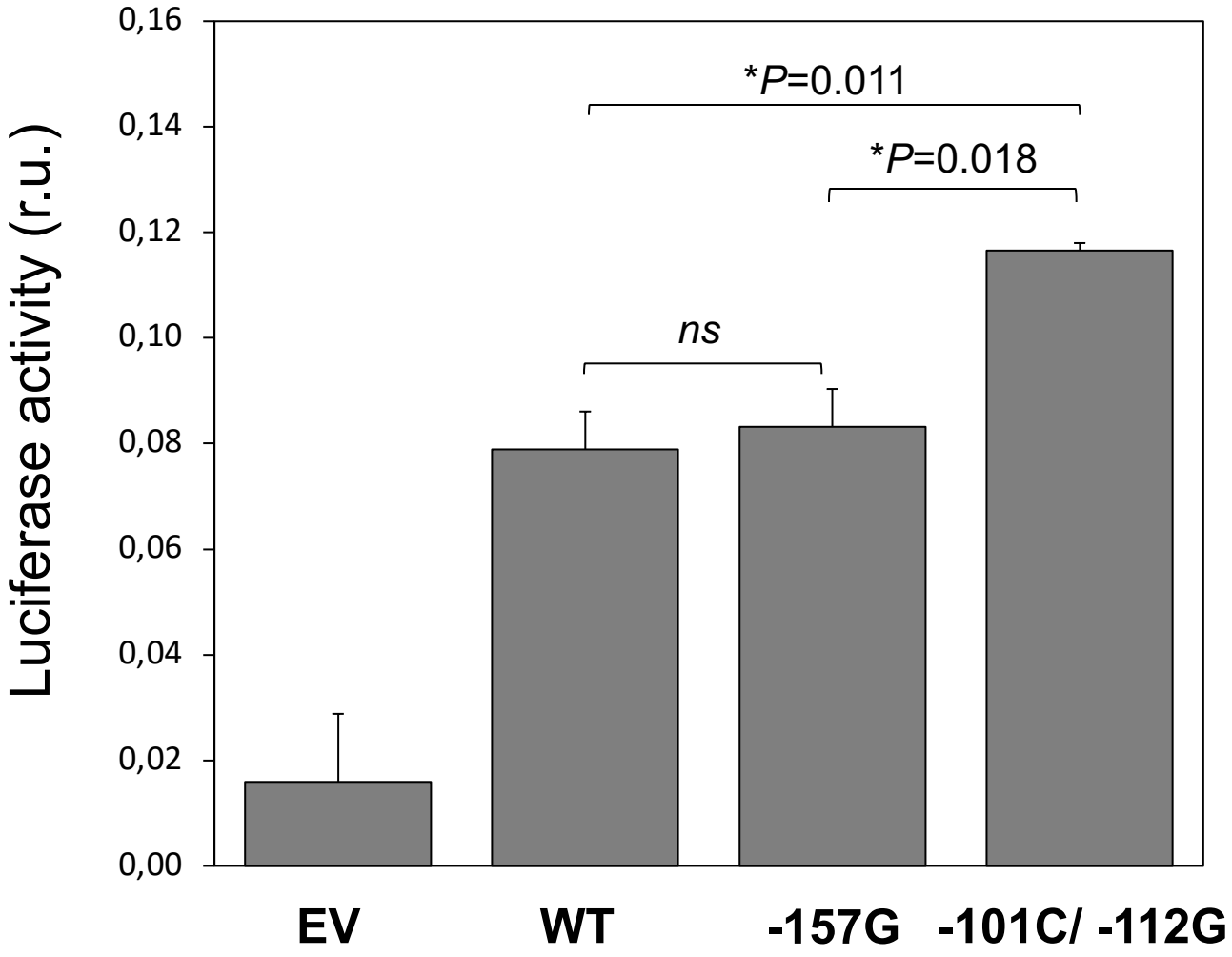
Luciferase activity (r.u.)
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
*P=0.011
*P=0.018
ns
EV
WT
-157G
-101C/ -112G

Figure 3

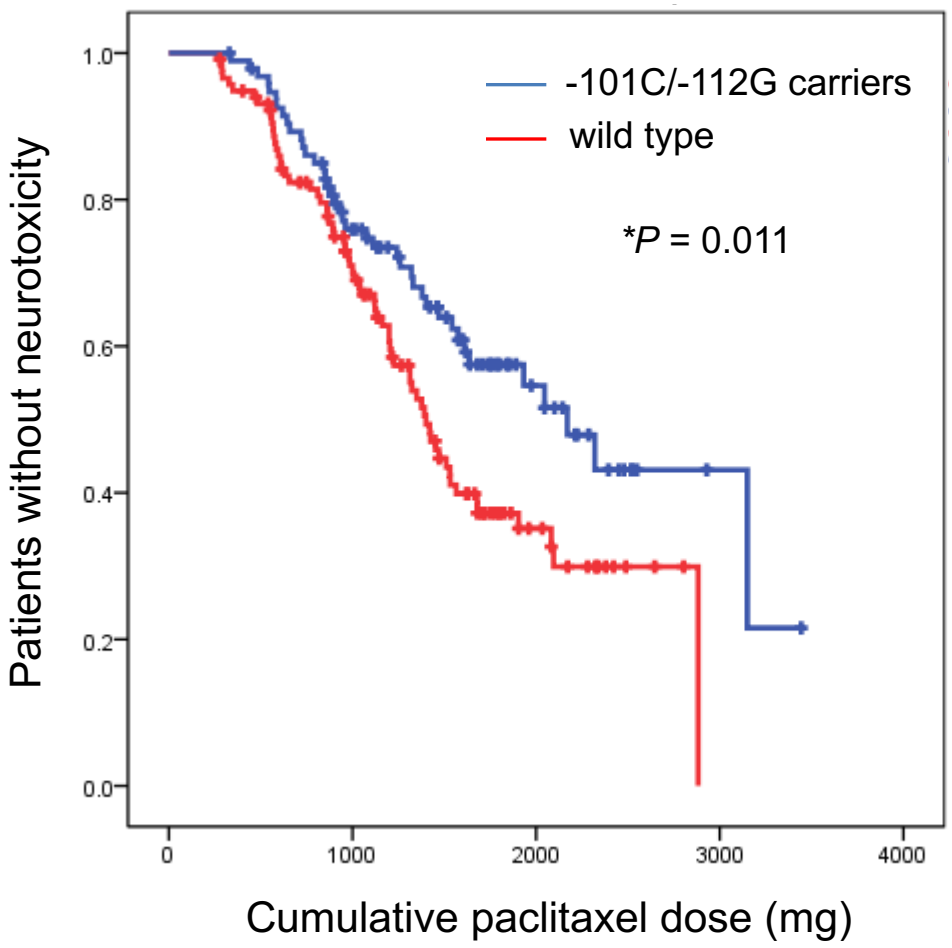
-101C/-112G carriers
wild type
*P = 0.011
Patients without neurotoxicity
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
Cumulative paclitaxel dose (mg)

Figure 4

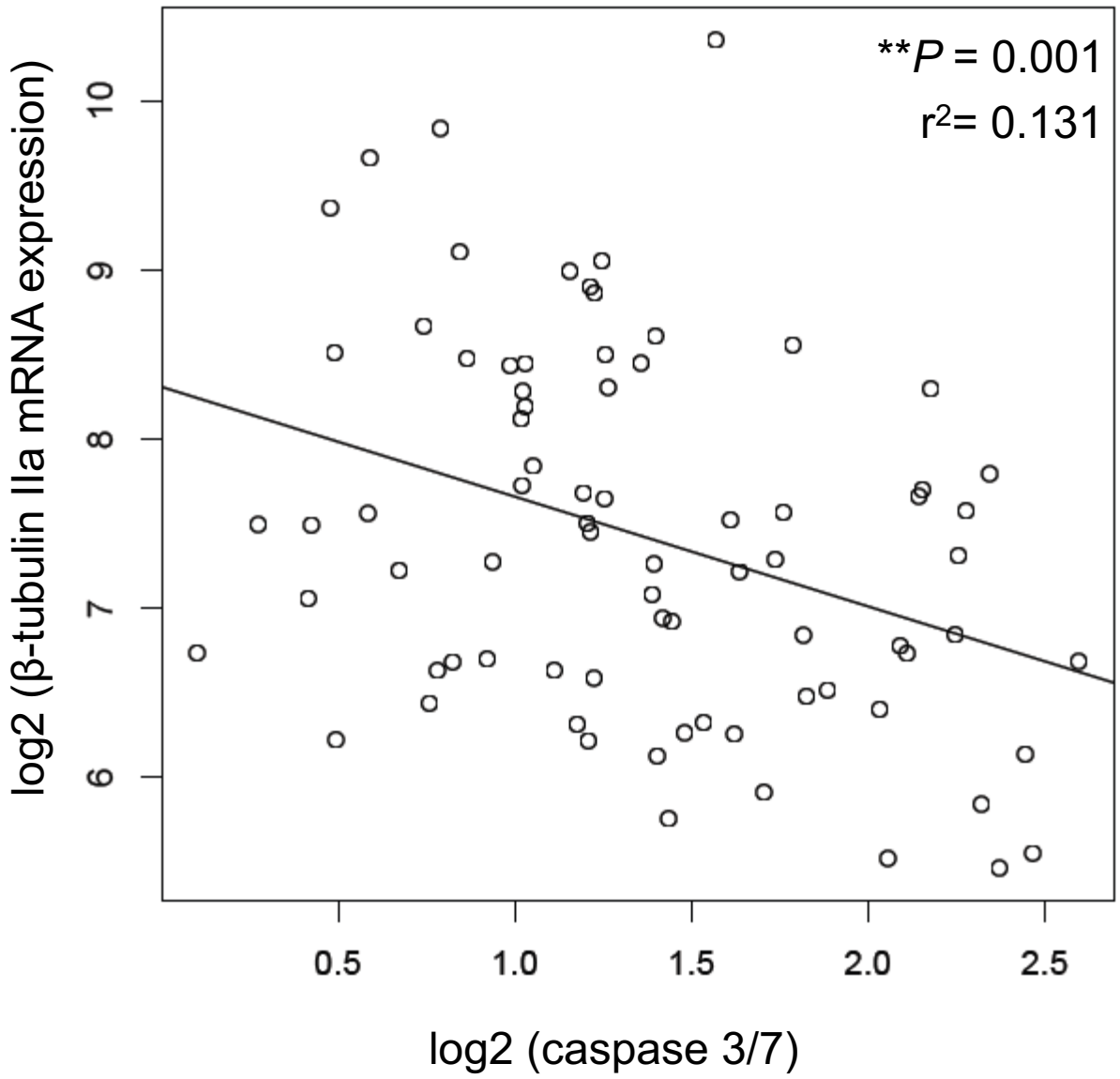
**P = 0.001
r2= 0.131
log2 (β-tubulin IIa mRNA expression)
6
7
8
9
10
0.5
1.0
1.5
2.0
2.5
log2 (caspase 3/7)

Table 1. Characteristics of the 214 patients included in the study.

Characteristics	No.	%
Age at study entry (years)		
Median	62	
IQR[a] (minimum–maximum)	69-56 (29–87)	
Gender		
Male	42	20
Female	172	80
Site of primary tumor		
Ovary	120	56
Lung	39	18
Breast	38	18
Other[b]	17	8
Chemotherapy[c]		
Paclitaxel 175+carboplatin	159	74
Paclitaxel 80	25	12
Paclitaxel 150+gemcitabine	7	3
Paclitaxel 90+bevacizumab	5	2
Paclitaxel 80+carboplatin	5	2
Paclitaxel 80+carboplatin+trastuzumab	4	2
Paclitaxel 175+cisplatin	3	1
Paclitaxel 80+cetuximab	2	1
Paclitaxel 80+trastuzumab	2	1
Paclitaxel 175+lapatinib	1	0.4
FAC–FEC followed by paclitaxel 80	1	0.4
Neurotoxicity[d]		
Grade 0	61	28
Grade 1	46	21
Grade 2	83	39
Grade 3	24	11
Treatment modification[e]		
No change	167	78
Reduction	22	10
Suspension	25	12

[a] Interquartile range (IQR).

[b] Other sites of primary tumor were uterus, head and neck, bladder, urinary tract, germinal and peritoneal.

[c] Paclitaxel 80-90mgm^{-2} had mainly 1 h infusion and 150-175mgm^{-2} mainly 3 h infusion. All doses in mgm^{-2} if not specified otherwise. The different treatments consisted on: paclitaxel 175+carboplatin (paclitaxel 175; carboplatin AUC 6/3 weeks); paclitaxel 80 (paclitaxel 80/weekly); paclitaxel 150+gemcitabine (paclitaxel 150; gemcitabine 2500/2 weeks); paclitaxel 90+bevacizumab (paclitaxel 11, 81 and 151

dose; bevacizumab 10mgkg^{-1} 11 and 151 dose/4 weeks); paclitaxel 80+carboplatin (paclitaxel 80+carboplatin AUC 2/weekly); paclitaxel 80+carboplatin+trastuzumab (paclitaxel 80; carboplatin AUC 2; and trastuzumab 2mgkg^{-1} /weekly); paclitaxel 175+cisplatin (paclitaxel 175; cisplatin 90/3 weeks) in one patient paclitaxel was administered i.p.; paclitaxel 80+cetuximab (paclitaxel 80; cetuximab 250/weekly); paclitaxel 80+trastuzumab (paclitaxel 80; trastuzumab 2mgkg^{-1} /weekly); paclitaxel 175+lapatinib (paclitaxel 175/3 weeks; lapatinib1250mg per dose); and FAC–FEC followed by paclitaxel 80 (FAC/FEC followed by paclitaxel 80/weekly).

[d] Maximum neurotoxicity according to National Cancer Institute (NCI) Common Toxicity Criteria version 2.

[e] Modifications of the treatment because of paclitaxel-induced neurotoxicity.