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Genome-wide association study identifies ephrin type-A receptors implicated in paclitaxel-induced peripheral sensory neuropathy

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Keywords: paclitaxel, peripheral neuropathy, polymorphisms, genome-wide association study, ephrin type-A receptors

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ABSTRACT

Background: Peripheral neuropathy is the dose-limiting toxicity of paclitaxel, a chemotherapeutic drug widely used to treat solid tumors. This toxicity exhibits great inter-individual variability of unknown origin. The present study aimed to identify genetic variants associated with paclitaxel-induced neuropathy via a whole-genome approach.

Methods: A genome-wide association study (GWAS) was performed in 144 white European patients uniformly treated with paclitaxel/carboplatin and for whom detailed data on neuropathy was available. Per-allele SNP associations were assessed by Cox regression, modeling the cumulative dose of paclitaxel up to the development of grade 2 sensory neuropathy.

Results: The strongest evidence of association was observed for the ephrin type-A receptor 4 (*EPHA4*) locus (rs17348202, $P=1.0x10^{-6}$), and *EPHA6* and *EPHA5* were among the top-25 and 50 hits (rs301927, $P=3.4x10^{-5}$ and rs1159057, $P=6.8x10^{-5}$), respectively. A meta-analysis of *EPHA5*-rs7349683, the top marker for paclitaxel-induced neuropathy in a previous GWAS ($r^2=0.79$ with rs1159057), gave a hazard ratio (HR) estimate of 1.68 ($P=1.4x10^{-9}$). Meta-analysis of the second hit of this GWAS, *XKR4*-rs4737264, gave a HR=1.71 ($P=3.1x10^{-8}$). Imputed SNPs at *LIMK2* locus were also strongly associated with this toxicity (HR=2.78, $P=2.0x10^{-7}$).

Conclusions: This study provides independent support of *EPHA5*-rs7349683 and *XKR4*-rs4737264 as the first markers of risk of paclitaxel-induced neuropathy. In addition, it suggests that other *EPHA* genes, also involved in axonal guidance and repair following neural injury, as well as *LIMK2* locus, may play an important role in the development of this toxicity. The identified SNPs could form the basis for individualized paclitaxel chemotherapy.

INTRODUCTION

Paclitaxel is an antineoplastic drug frequently used in first-line treatment for breast, ovarian, lung and prostate cancers. This molecule binds the cellular microtubules through the β-tubulin subunit, promoting their stabilization, preventing cell division and finally leading to apoptosis. Although paclitaxel is an effective treatment for various types of cancers, there are associated toxicities that lead to serious clinical limitations in its use. Peripheral neuropathy is the dose-limiting toxicity of paclitaxel, with most patients treated with the drug developing this adverse effect [1]. Peripheral neuropathy is predominantly sensory and is generally axonal, distal, symmetrical, debilitating and painful [2]. Although the causal mechanisms have not been well defined, it is clear that microtubule-mediated axonal transport is affected [3]. Paclitaxel neurotoxicity is dose-dependent and there are various clinical factors that have been suggested to increase the risk of developing it, such as pre-treatment with neurotoxic agents, use of anti-retroviral drugs, and a personal history of *diabetes mellitus*, chronic liver disease, alcoholism, hypothyroidism, nutritional deficits or hereditary or acquired polyneuropathies [1]. However, despite these factors, there is great inter-individual variability in the neurotoxicity of patients receiving similar amounts of paclitaxel under similar protocols, with some patients suffering serious neuropathies which lead to dose reductions and suspensions. Peripheral neuropathy usually takes months to disappear, and in the most severe cases the damage to the peripheral nerves can be irreversible. Therefore, the identification of biomarkers to predict the risk of suffering severe neuropathy would be of great clinical utility.

Our group and others have applied candidate gene approaches, focused on the pharmacokinetic and pharmacodynamic pathways of paclitaxel, to identify genetic variants associated with peripheral neuropathy, in some cases observing contradictory results. In any case, the variants identified explain only a relatively small fraction of the variation in

neuropathy outcome [4, 5, 6, 7, 8]. Recently, the first genome-wide association study (GWAS) of paclitaxel-induced neuropathy was published, and identified SNPs in genes not previously studied, such as *EPHA5, XKR4* and *FGD4* [9], as putative markers of risk of this toxicity. However, replication of these results and/or meta-analyses including other GWAS are required to provide sufficient evidence to consider them to guide clinical use of this critically important chemotherapeutic agent. In this study we performed a GWAS with 144 white European cancer patients homogenously treated with paclitaxel/carboplatin and for whom detailed neuropathy data were collected. We provide independent support of *EPHA5*-rs7349683 and *XKR4*-rs4737264 as the first markers of paclitaxel-induced peripheral neuropathy and suggest common variants in other *EPHA* genes, and at *LIMK2* locus, as potential additional markers of susceptibility to this toxicity.

MATERIALS AND METHODS

Patients and peripheral neuropathy assessment

Blood or saliva samples were collected from 144 cancer patients treated with paclitaxel in one Spanish and two Swedish centers: 48 patients (33%) from Hospital Universitario Fundación Alcorcón [7], 63 (44%) from Karolinska University Hospital, and 33 (23%) from Linköping University Hospital [10]. Eligible patients were over 18 years of age and had: received a chemotherapy regimen with paclitaxel 175mg/m^2 and carboplatin AUC 5-6 every 21 days to treat a histologically documented solid neoplasia; life expectancy of ≥12 weeks; Eastern Cooperative Oncology Group performance status of 2 or less; no chemotherapy, hormonal therapy nor radiotherapy in the 4 weeks prior to treatment; taken contraception (fertile women only); adequate bone marrow, renal and hepatic function; and no previous history of neuropathy. Most patients had ovary or lung malignancies (70% and 19%, respectively) and paclitaxel/carboplatin chemotherapy was administered as first-line treatment in the vast majority (94%, Table 1). All patients were of white European origin. The collection of samples was approved by local Internal Ethical Review Committees and all patients gave written informed consent. Additional details related to the patients, tumors and treatments are summarized in Table 1.

Table 1. Patient characteristics.

Characteristic	No.	%
Age at treatment (years)		
Median (range)	63 (34-82)	
Interquartile range	57-69	
Gender		
Male	21	15
Female	123	85
Site of primary tumor		
Ovary	101	70
Fallopian tube	8	6
Peritoneum	5	3
Lung	27	19
Uterus	2	1
Breast	1	1
Tumor stage		
I	15	10
II	8	6
III	83	58
IV	38	26
Paclitaxel+Carboplatin treatment[a]		
Median number cycles (range)	6 (2-9)	
Chemotherapy administered previously	9	6
Neurotoxic drugs administered previously[b]	6	4

[a] Paclitaxel 175mg/m^2 + carboplatin 5-6 AUC every 21 days.

[b] Median of 34 weeks prior to paclitaxel chemotherapy (range 4-364 weeks).

Demographic, tumor and treatment data were prospectively collected by medical chart review and stored in an electronic database. To guarantee that information about neuropathy was collected in a homogenous manner, the three participating centers designed a common questionnaire based on the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2 which included details of number of treatment cycles, sensory symptoms such as paresthesia (in the feet only, or present in both the feet and hands), and functionally disabling

neuropathies. Although a more extensive assessment of sensory and motor paclitaxel-induced neurotoxicity was undertaken for a subset of 71 patients [7, 11] (Supplementary Methods), only NCI-CTC scoring was used for the analyses. Neuropathy symptoms at baseline and cumulative paclitaxel dose at first neuropathy event, at grade 2 sensory neuropathy and at maximum neuropathy grade were also collected from all patients. Baseline neuropathy was recorded as zero, when the patients did not report symptoms of neuropathy previously to starting the chemotherapy. In addition, details of treatment delays, dose reductions and treatment suspensions, and the reasons for these, were recorded, together with the cumulative paclitaxel dose at any changes in treatment (see Table 2).

Table 2. Paclitaxel-induced peripheral neuropathy.

Origin	Cumulative paclitaxel dose up to grade 2 sensory neuropathy		Dose reductions due to neuropathy		Dose suspensions due to neuropathy	
	median (mg)	median (mg/m^2)	No.	%	No.	%
Total (n=144)	2046	1225	19	13	17	12
Sweden (n=96)	2083	1225	15	16	12	13
Spain (n=48)	1733	1050	4	8	5	10

DNA isolation, genotyping, quality control and SNP imputation

DNA was isolated from peripheral blood and from saliva using standard protocols. The final DNA concentration was quantified by PicoGreen (Invitrogen, Carlsbad, USA). Genotyping was performed on 250 ng of DNA using the Infinium BeadChip Human 660WQuad assay (Illumina, San Diego, USA) which consists of 657,366 markers in a 4-sample array format. The GenomeStudio software package was used to extract genotype data from files created by the Illumina iScan System. One sample with a call rate <0.95, probably due to poor DNA quality, was excluded; all other samples had call rates >0.99. Non-diploid variants (e.g. mitochondrial chromosomes), CNV probes and SNPs deemed unreliable by Illumina (Tech Note: Infinium® Genotyping Data Analysis, 2007) were excluded, leaving

559,348 SNPs. After also excluding SNPs with missing genotypes in more than 5% of samples, as well as those with minor allele frequency (MAF) <0.025, 518,577 SNPs were included in the association analysis. Genotyping for rs5749248 was performed using the KASPar SNP genotyping system (Kbiosciences, Hoddesdon, UK) and fluorescence was determined and alleles assigned by the sequence Detection System 7900HT (Applied Biosystems, Foster City, USA).

Genotypes were imputed for additional SNPs in or near *EPHA4, EPHA6, EPHA5, EPHA8* and *LIMK2* based on data from the 1000 Genomes Project (June 2011 release) using IMPUTE (version 2.0). Prior to imputation, genotyped SNPs were filtered and limited to those that were autosomal, with MAF ≥0.01 and P-value for departure from Hardy Weinberg Equilibrium (HWE) ≥0.001. Imputed SNPs with MAF <0.025 and call rate <0.90 were excluded.

Statistical analysis

Associations with risk of paclitaxel-induced sensory neuropathy were tested for clinical factors and SNPs using Cox regression analysis, modeling the cumulative dose of paclitaxel up to the development of grade 2 peripheral sensory neuropathy. Patients with no or minimal adverse reaction (grade 0/1) were censored at total paclitaxel cumulative dose (mg). Associations with SNPs were assessed under an additive genetic model by multivariable analysis; age was included as a covariate because there was weak evidence that older patients were at increased risk of sensory neuropathy (HR per year=1.02; CI95%=1.00-1.05; P=0.082). Although motor neuropathy was also recorded in the database, its incidence is lower than the sensory neuropathy (i.e. only 6% of the patients developed motor neuropathy grade 2 or higher; while 48% of the patients developed sensory neuropathy grade 2 or higher) and it was not analyzed due to low statistical power. Cox regression analyses were performed

using the R statistical software (version 2.14.0); all other statistical analysis were carried out using PLINK (version 1.07). All SNPs with a nominal P-value for association $\leq 10^{-4}$ were further evaluated for potential errors by visualizing genotype cluster plots, comparing the estimated MAF with that reported by the HapMap Project for the CEU population (using Pearson´s χ^2 test) and considering failure rates and evidence for departure from HWE; details are provided in Table 3. All SNPs except one (P>0.005) had P-values >0.01 for departure from HWE, and none had missing genotypes. To account for possible differences in the ethnic origin of patients, analyses were repeated adjusting for country of origin, with no substantial differences observed in the results obtained. Also, using prior chemotherapy as an additional covariate in the analysis did not have an impact on the results (data not shown). Statistical analyses were also performed using cumulative paclitaxel doses in mg/m^2 without finding substantial changes in the results obtained. The meta-analysis was performed using the R package *meta* in a fixed effect model, because of the similarities between studies (same phenotype measured on the same scale, ethnicity and genetic effects) and because both Cochran's Q measure of heterogeneity and I^2 statistic showed low variation between them. Haplotype analysis was conducted for *LIMK2* region using genotyped SNPs. Haplotype blocks were identified in HapMap CEU samples using the Haploview v 4.2 software, based on the method described by Gabriel *et al*. [12], and haplotypes were imputed using PHASE v2.0. The association of each haplotype with peripheral neuropathy was assessed using Cox regression, adjusted for age.

RESULTS

The distribution of paclitaxel-induced peripheral sensory neuropathy in the included patients is shown in Table 2. The median cumulative paclitaxel dose at which patients developed peripheral sensory neuropathy was 2,046 mg and this toxicity caused dose modifications in 25% of patients. There was no significant difference in the incidence of neuropathy between Spanish and Swedish patients (P=0.91). No association with paclitaxel-induced sensory neuropathy was observed for gender, tumor type, tumor stage, previous chemotherapy or prior use of neurotoxic drugs ($P \geq 0.29$).

None of the SNPs genotyped in the array were associated with risk of sensory neuropathy at genome-wide statistical significance (defined as $P<5x10^{-7}$ [13]), but P-values $<10^{-5}$ were observed for several SNPs (Table 3). The strongest evidence of association was observed for rs17348202 (HR=4.85, 95%CI=2.57-9.13, $P=1.02x10^{-6}$; Table 3 and Fig. 1A), located downstream of *EPHA4*. Imputation of additional SNPs at the *EPHA4* locus did not suggest any stronger association signals (data not shown).

Table 3. Twenty-five SNPs with strongest association with grade 2 paclitaxel-induced peripheral sensory neuropathy.

SNP[a]	Chromosome	Gene[b,c]	Major allele	Minor allele	MAF[d]	HR (95% CI)[e]	*P*-value[e]
rs17348202	2	*EPHA4*	A	G	0.05	4.85 (2.57-9.13)	1.02×10^{-6}
rs4141404	22	***LIMK2*** (*PLA2G3-PISD*)[f]	C	A	0.25	2.41 (1.66-3.48)	3.22×10^{-6}
rs275456	5	*PAPD7*	C	A	0.24	2.26 (1.60-3.18)	3.31×10^{-6}
rs1165472	1	***RP11-466L17.1***	A	G	0.30	2.36 (1.64-3.40)	3.65×10^{-6}
rs3181157	12	*CD9*	G	A	0.10	3.22 (1.96-5.29)	4.05×10^{-6}
rs10090117	8	*PSD3/ NAT2*	A	G	0.21	2.38 (1.64-3.44)	4.23×10^{-6}
rs10065203	5	***TRIO***	G	A	0.40[*]	2.51 (1.69-3.71)	4.25×10^{-6}
rs2947253	15	*ATPBD4/ RP11-702M1.1*	*A*	G	0.11	3.38 (2.01-5.68)	4.36×10^{-6}
rs10512385	9	*ACTL7B*	A	G	0.14	2.58 (1.71-3.89)	5.70×10^{-6}
rs12699683	7	*AGMO/ DGKB*	C	A	0.09	3.66 (2.08-6.45)	6.65×10^{-6}
rs501461	9	***GLIS3***	A	C	0.39	0.43 (0.29-0.63)	1.24×10^{-5}
rs6846708	4	***PALLD***	A	G	0.07	3.64 (2.03-6.53)	1.50×10^{-5}
rs2425553	20	***PTPRT***[g]	G	A	0.21	2.33 (1.58-3.44)	1.94×10^{-5}
rs1753097	13	***SGCG***	A	G	0.20	2.18 (1.52-3.11)	1.96×10^{-5}
rs6473187	8	***KIAA0146***-*PRKD*[h]	A	G	0.06	3.37 (1.92-5.91)	2.17×10^{-5}
rs13163920	5	*PAPD7*	G	A	0.27	2.41 (1.60-3.61)	2.23×10^{-5}
rs10932374	2	***ERBB4***	G	A	0.26	2.25 (1.54-3.28)	2.58×10^{-5}
rs3829306	12	***SLCO1B1***[i]	G	A	0.09	3.10 (1.82-5.26)	2.84×10^{-5}
rs8110536	19	***C19orf21***	A	C	0.17	2.24 (1.53-3.27)	2.98×10^{-5}
rs189372	3	*LPP*	G	A	0.39	2.27 (1.55-3.35)	2.98×10^{-5}
rs7655560	4	*HAND2*	G	A	0.06	2.97 (1.78-4.95)	3.27×10^{-5}
rs9365397	6	***PARK2***	A	G	0.04	4.33 (2.16-8.65)	3.42×10^{-5}
rs301927	3	***EPHA6***	A	G	0.17	2.35 (1.57-3.53)	3.44×10^{-5}
rs2413045	22	***LIMK2***	G	A	0.15	2.36 (1.57-3.56)	3.67×10^{-5}
rs12743802	1	*TYW3/ LHX8*	G	A	0.04	4.06 (2.08-7.91)	3.85×10^{-5}

[a] For pairs of SNPs with $r^2 > 0.6$, only the SNP with the lowest P-value is shown.
[b] Intergenic SNPs are denoted by the closest flanking annotated gene(s).
[c] Bold type indicates that the SNP is located in the gene.
[d] The MAF was compared with that reported in the HapMap Project CEU population using Pearson´s Chi-square test. MAF differences with P<0.01 are marked with an asterisk.
[e] Estimated by multivariable Cox regression analysis adjusted for age.
[f] rs4141404 is located in *LIMK2*, but there is a large region of linkage disequilibrium (LD) (r^2>0.7) that covers several genes, from *PLA2G3* to *PISD*.
[g] rs2425553 is in complete LD with rs2425556 and rs3092292.
[h] rs6473187 in *KIAA0146* is in complete LD with rs2632496 in *KIAA0146*, with rs4873774 in *UBE2V2* and with rs8178108 in *PRKD*.
[i] rs3829306 is in complete LD with rs4149013 and rs4149023, both in *SLCO1B1*.

MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval.

Interestingly, a synonymous SNP in *EPHA5* was the top association hit in a previous GWAS of paclitaxel-induced neuropathy (rs7349683, HR=1.63, 95%CI=1.34-1.98, P=$9.6x10^{-7}$) [9]. In our GWAS, two SNPs in complete LD, rs1159057 and rs12507286, located in a ~50 kb LD block that included the *EPHA5* gene, were among the top 50 hits based on P-value (HR=2.01, 95%CI=1.43-2.84, P=$6.84x10^{-5}$; Table 4). SNP imputation revealed rs139491476 as having a slightly stronger association signal (P=$3.95x10^{-5}$, Suppl. Fig. 1). The previously reported *EPHA5* marker, rs7349683, in high LD with rs1159057 (r^2=0.79), had a P-value for association of $3.33x10^{-4}$ in the present study (HR=1.83, 95%CI=1.32-2.55; Fig. 1B; Suppl. Fig. 1). A meta-analysis of results for rs7349683 from both GWAS gave a genome-wide statistically significant P-value of $1.4x10^{-9}$ (HR=1.68, 95%CI=1.42-1.99).

Table 4. SNPs in EPH type A receptors associated with grade 2 paclitaxel-induced peripheral sensory neuropathy.

Gene	SNP[a]	Location	Major allele	Minor allele	MAF	HR (95% CI)[b]	P-value[b]
EPHA4	rs17348202	210 kb downstream	G	A	0.05	4.85 (2.57-9.13)	1.02×10^{-6}
EPHA6	rs301927	in gene (intronic)	G	A	0.17	2.35 (1.57-3.53)	3.44×10^{-5}
EPHA5	rs1159057[c]	48 kb downstream, LD covers gene	A	G	0.44	2.01 (1.43-2.84)	6.84×10^{-5}
EPHA8	rs209709	12 kb upstream	G	A	0.17	2.20 (1.36-3.55)	1.28×10^{-3}

[a] Genotyped SNPs in *EPHA* genes associated with neuropathy at P<0.005. SNPs shown in the Table are those with the lowest P-value for each gene.

[b] Estimated by multivariable Cox regression analysis adjusted for age.

[c] rs1159057 is in complete LD with rs12507286.

MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval.

Given that our and the previous GWAS observed the strongest signals for *EPHA4* and *EPHA5*, respectively, and that the EphA family is involved in nerve damage response [14, 15, 16, 17, 18], we examined more closely the results for SNPs in genes from this family. An intronic SNP in *EPHA6*, rs301927, was among the 25 top hits (HR=2.35, 95%CI=1.57-3.53, $P=3.44x10^{-5}$; Table 3, Fig. 1C). Imputation of further SNPs at this locus revealed 4 SNPs, in complete LD, with a lower P-value ($P=2.87x10^{-5}$, Suppl. Fig. 2). In addition, rs209709, 12 kb upstream of *EPHA8*, had a P-value for association of $1.28x10^{-3}$ (Table 4) and imputation revealed an *EPHA8* intronic SNP, rs3754005, and a missense variant, rs606002, not in LD with rs209709 ($r^2<0.03$), with P-values of $9.83x10^{-4}$ and $3.36x10^{-3}$, respectively. No other SNPs in *EPHA* genes appeared to be associated with paclitaxel-induced neuropathy.

The SNP with second-lowest P-value for association was rs4141404 (HR=2.41; 95%CI=1.66-3.48, $P=3.22\ x10^{-6}$; Table 3), located in the 3'UTR of *LIMK2* gene. Analysis of the surrounding LD structure for the CEU population from the 1000 Genomes Project revealed a ~500 kb region with $r^2>0.7$ including 8 additional genes (Suppl. Fig. 3). An intronic SNP in *LIMK2*, rs2413045, but in relatively low LD with rs4141404 ($r^2=0.58$), was also among the top 25 hits (Table 3). SNP imputation identified two SNPs (rs5749227 and rs5749248, in complete LD) that showed a stronger association with neuropathy ($P=6.38x10^{-8}$, see Suppl. Fig. 3). Direct genotyping of rs5749248 confirmed an association with paclitaxel-induced peripheral neuropathy that reached genome-wide statistical significance (HR=2.78, 95%CI=1.89-4.08, $P=1.98x10^{-7}$, Fig. 1D). Haplotype analysis of SNPs at this locus did not suggest any stronger associations with neuropathy than the single SNP analysis (data not shown).

In addition, it is interesting to note that rs3829306, rs4149023 and rs4149013 were among the 25-top associated SNPs. These SNPs in complete LD are located on introns and

upstream of *SLCO1B1*, the gene encoding the paclitaxel hepatic up-take transporter OATP1B1 (Table 3).

With respect to paclitaxel-induced neuropathy markers proposed by Baldwin *et al.* [9], we performed a meta-analysis of results for their 8 top SNPs (Supplementary Table 1). In addition to rs7349683 in *EPHA5*, Baldwin's second hit rs4737264 in *XKR4* gave a genome-wide statistically significant P-value of 3.11×10^{-8} (HR=1.71; 95%CI=1.41-2.06).

DISCUSSION

We have performed a genome-wide association study to identify genetic variants associated with paclitaxel-induced peripheral sensory neuropathy, the dose-limiting toxicity of this drug. These polymorphisms might serve as markers to define subsets of patients at high risk of neuropathy that could receive alternative chemotherapeutic regimens. Neuropathy can decrease not only the quality of life of patients but may also alter the efficacy of treatment, through early dose reductions and suspensions. Several candidate-gene association studies, have given conflicting results [4, 6, 7, 8, 19], and a major part of the inter-individual variability in paclitaxel-induced neuropathy remains unexplained. A recently published GWAS on this toxicity, based on the clinical trial CALGB 40101, identified putative novel susceptibility loci [9], although replication in independent studies is required to confirm these. Here we independently replicate the association of *EPHA5* and *XKR4* with paclitaxel-induced peripheral sensory neuropathy reported by Baldwin *et al*, and propose additional genetic susceptibility loci in other *EPHA* genes and at *LIMK2* locus.

There are nine EphAs in humans that are expressed in almost all tissue types during development and in most cell types of adults. EphA/ephrin-A signaling is crucial for nervous system development, tissue regeneration and tumor progression [20]. *EphA5* knockout mice have shown that this receptor is essential in the initiation of the early phases of synaptogenesis [21], with EphA5 expression increasing in response to sciatic nerve lesions [14]. EphA4 is directly implicated in the regulation of axonal regeneration [15, 16], astrocyte responsiveness [22] and other pathways involved in the repair of neural injury [17]. In addition, recent studies of amyotrophic lateral sclerosis identified EphA4 as a determinant of the vulnerability of neurons to axonal degeneration [18]. *EphA6* knockout mice were found to have behavioral deficits as well as learning and memory impairment [23], and findings in *EphA8* knockout mice suggest that this receptor plays a role in axonal path-finding during the

development of the mammalian nervous system [24]. Taken together, these results highlight the relevant function of EphAs in neurons and in pathways involved in the repair of neural injury.

In this study we replicate the finding that the *EPHA5* synonymous variant rs7349683 is a marker of risk of paclitaxel-induced neuropathy; with the association reaching genome-wide statistical significance in the meta-analysis ($P=1.4x10^{-9}$) with an estimated HR of 1.68 (95% CI=1.42-1.99). Baldwin *et al* also included in their study a small replication set in which rs7349683 was genotyped; inclusion of results from this in the meta-analysis gave a HR estimate of 1.59 (95% CI=1.36-1.87) with a P-value of $1.1x10^{-8}$. Approximately 48% and 16% of patients will be heterozygous and homozygous, respectively, for the rare allele of rs7349683, and have an estimated 1.68- and 2.82-fold higher risk of neuropathy, respectively, than wild-type homozygous patients. Interestingly, rs17348202 and rs301927, our 1st and 23rd top hits, are also located in or near *EPHA4* and *EPHA6*, respectively, and a SNP near *EPHA8* showed weaker evidence of association (Table 4). On the whole, these findings suggest that polymorphisms in the *EPHA* genes may play a crucial role in the development of paclitaxel-induced neuropathy and should therefore be studied further.

When we examined other paclitaxel-induced neuropathy markers proposed by Baldwin *et al.* [9], in addition to rs7349683 in *EPHA5*, rs4737264 in *XKR4* also gave a genome-wide statistically significant P-value in the meta-analysis ($P=3.11x10^{-8}$). This is an intronic SNP in *XKR4*, a still uncharacterized protein expressed in cerebellum, previously associated with attention deficit/hyperactivity disorder [25] and to iloperidone and risperidone response [26, 27]. Among the top-25 most statistically significant associations were two independent SNPs located at *LIMK2* locus (Table 3), and direct genotyping of the imputed SNP rs5749248 confirmed a genome-wide statistically significant association ($P=1.98x10^{-7}$, Fig. 1D). *LIMK2* knockout mice exhibit minimal abnormalities, but *LIMK-1/2* double-knockout mice have

impaired excitatory synaptic function [28]. In addition, knockdown of *LIMK2* in cell lines results in significantly reduced neurite-bearing cell count, neurite length and cone extension growth rate [29]. Furthermore, *LIMK2* expression has been implicated in sensitivity to the microtubule-binding agents vincristine and vinblastine in a neuroblastoma cell line [30]. With respect to other genes at this locus, *PIK3IP* is a negative regulator of PI3K and *PLA2G3* is involved in oxidative stress and is associated with Alzheimer's disease.

It is important to highlight that the 25 hits with strongest evidence of association also included *SLCO1B1*, the gene encoding OATP1B1, which is a paclitaxel hepatic up-take transporter previously studied in paclitaxel-related candidate gene approaches [7, 31]. The OATP1B1 amino acid change V174A, caused by rs4149056, is known to decrease the transport of several drugs including docetaxel [32]. However, this variant is not correlated with rs3829306 (r^2=0.012) and is not associated with neuropathy (HR=1.41, 95%CI=0.95-2.09, P=0.089).

As discussed previously, we have independently replicated two associations from a previous GWAS, confirming *EPHA5*-rs7349683 and *XKR4*-rs4737264 as markers of paclitaxel-induced neuropathy [9]. However, we did not observe evidence of association for other top hits from that study, such as *FGD4* and *FZD3*. These discrepancies could be due to differences between the two studies. While Baldwin *et al.´*s GWAS was based on 855 breast cancer patients treated with paclitaxel monotherapy at 175 mg/m^2 every 2 weeks, we included mostly ovarian and lung cancer patients treated with paclitaxel 175 mg/m^2 plus carboplatin AUC 5-6 every 3 weeks. We did not find differences in the incidence of neuropathy among the different types of tumors included, but breast cancer was not considered. Both GWAS included patients with European ancestry and the associations with risk of neuropathy were assessed using Cox regression, modeling the cumulative dose of paclitaxel up to the development of grade 2 peripheral sensory neuropathy. It is important to highlight the

inaccuracy of neuropathy assessment based exclusively on NCI-CTC grading reported by physicians [33]. We applied the same criterion to assess neuropathy in the 3 participating hospitals, and half of the patients went through a thorough neurologic examination [7, 10]. However, the limited number of samples included in our study implies reduced statistical power, making it possible that additional true associations went undetected. It is also important to highlight that since all of our patients were treated with a combination of paclitaxel and carboplatin, even though paclitaxel is more neurotoxic than carboplatin [34], we cannot affirm that the neuropathy markers found correspond only to paclitaxel and not carboplatin or a combination of both drugs. Despite these possibilities and the differences between the studies, both GWAS found an association for *EPHA5*-rs7349683 and *XKR4*-rs4737264, thus indicating that these are neuropathy markers valid for the different paclitaxel chemotherapy regimens considered. In addition, the nerve injury repair function of EphA5 suggests that this marker could also be valid for other neurotoxic drugs that depend on repair pathways similar to those of paclitaxel [35, 36]. Altogether, the results obtained by our group and others, suggest that several common variants associated with paclitaxel sensorial peripheral neuropathy with moderate effect sizes are expected. The combination of these markers would improve the genetic prediction capability for this toxicity, facilitating implementation in the clinic. To this respect, different polygenic modeling methods have been proposed to explain a larger proportion of a trait under study [37, 38, 39].

In summary, this study confirms *EPHA5*-rs7349683 and *XKR4*-rs4737264 as markers of risk of paclitaxel-induced sensory neuropathy. In addition, it suggests that common variants in other *EPHA* genes and at the *LIMK2* locus could also play a role in this toxicity. Together, these findings suggest that genes involved in the function and repair of peripheral nerves, which had not previously been studied in candidate gene approaches, could make a substantial contribution to the genetic susceptibility to this toxicity. *EPHA5*-rs7349683 and

XKR4-rs4737264 appear to be the first markers which, when combined with others as they emerge, could be used clinically to classify patients according to their neuropathy risk, an important step towards individualized paclitaxel chemotherapy.

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COMPETING INTERESTS

None.

PATIENT CONSENT

Obtained.

ETHICS APPROVAL

The local ethics committees in Spain and Sweden.

CONTRIBUTORS

LJL-G, EA-L, HG and CR-A designed the study. LJL-G, EH, SL, CJ, XM, EA-L, HR and CR-A did the data collection. LJL-G, LI-P, GP, AG-N, MR, EA-L, HG and CR-A did the data analysis, data interpretation, and wrote the manuscript. CJ, EA-L and HG recruited patients. All authors critically reviewed the manuscript and approved the final version.

PROVENANCE AND PEER REVIEW

Not commissioned; externally peer reviewed

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FIGURE LEGENDS

Figure 1. Kaplan–Meier comparisons of cumulative dose of paclitaxel up to the development of grade 2 peripheral sensory neuropathy, by genotype for SNPs in the *EPHA4, LIMK2, EPHA6* and *EPHA5* loci. Patients treated with paclitaxel were grouped according to genotypes for **A)** rs17348202, **B**) rs5749248, **C)** rs301927 and **D)** rs7349683.

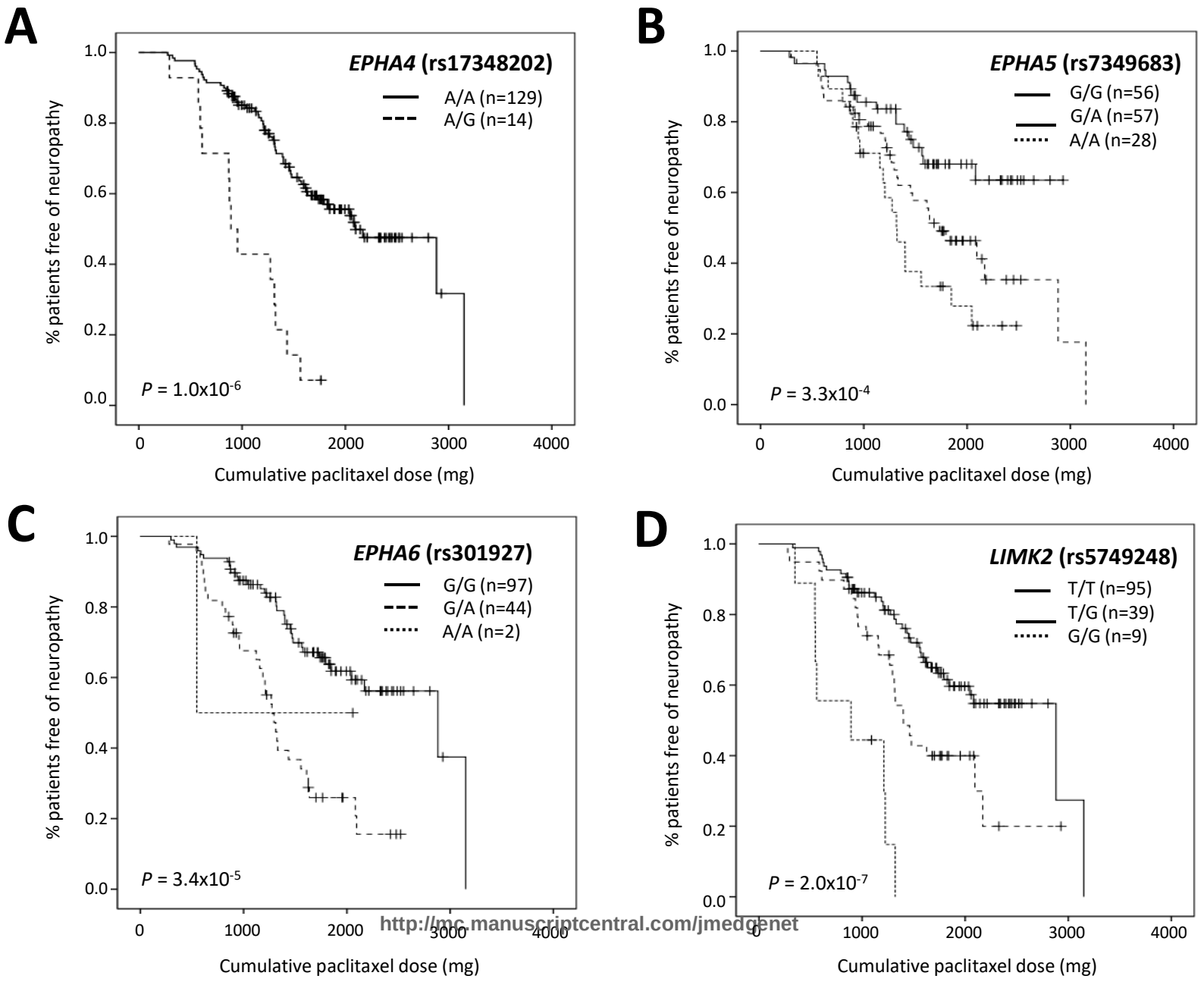
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Regional association plot for the *EPHA5* locus based on P-values for genotyped and imputed SNPs surrounding rs1159057. Imputed SNPs are shown as circles, SNPs genotyped on the HumanHap660-Quad BeadChip as triangles, and rs1159057 as a diamond. Recombination rates from HapMap (centimorgans per megabase) are plotted to reflect the local LD structure. Genes are shown in the lower panel of the plot. SNPs are colored to reflect their LD with rs1159057, based on pairwise r^2 values from the 1000 Genomes Project (Nov 2010) CEU; the r^2 value for rs139491476 was not available from this database, and so it was estimated using our own set of samples. The figure was generated using LocusZoom.

Supplementary Figure 2. Regional association plot for the *EPHA6* locus based on P-values for genotyped and imputed SNPs surrounding rs301927. Imputed SNPs are shown as circles, SNPs genotyped on the HumanHap660-Quad BeadChip as triangles, and rs301927 as a diamond. Recombination rates from HapMap (centimorgans per megabase) are plotted to reflect the local LD structure. Genes are shown in the lower panel of the plot. SNPs are colored to reflect their LD with rs301927, based on pairwise r^2 values from the 1000 Genomes Project (Nov 2010) CEU. The figure was generated using LocusZoom.

Supplementary Figure 3. Regional association plot for the *LIMK2* locus based on P-values for genotyped and imputed SNPs surrounding rs4141404. Imputed SNPs are shown as circles, SNPs genotyped on the HumanHap660-Quad BeadChip as triangles, and rs4141404 as a diamond. Recombination rates from HapMap (centimorgans per megabase) are plotted to reflect the local LD structure. Genes are shown in the lower panel of the plot. SNPs are colored to reflect their LD with rs4141404, based on pairwise r^2 values from the 1000 Genomes Project (Nov 2010) CEU. The figure was generated using LocusZoom [40].

Figure 1



SUPPLEMENTARY METHODS

Additional assessment of sensory and motor paclitaxel-induced neurotoxicity

A more extensive assessment was undertaken for a subset of 71 patients: 48 from Hospital Universtario Fundación Alcorcón (Leskelä *et al*. Pharmacogenomics J, 2011, 11, 121-129) and 23 from Linköping University Hospital (Green et al., Basic Clin Pharmacol Toxicol 2009, 104, 130-137).

With respect to the neurologic assessment of the 48 Spanish patients, sensory neuropathy grade 1 included numbness/paresthesia in the feet, in grade 2 theses symptoms were present in both fingers and feet, and grade 3 consisted of functional disabling numbness/paresthesia. Motor neuropathy grade 1 was defined by weakness in feet, in grade 2 the symptoms were present in both extremities and patients with grade 3 had trouble walking. On the first day of each chemotherapy cycle an evaluation of neurologic symptoms (sensory symptoms such as numbness and tingling in fingers of hands and feet, difficulty feeling the shape of objects, and motor symptoms such as general weakness, trouble walking and trouble buttoning buttons) and a physical and neurological examination was carried out.

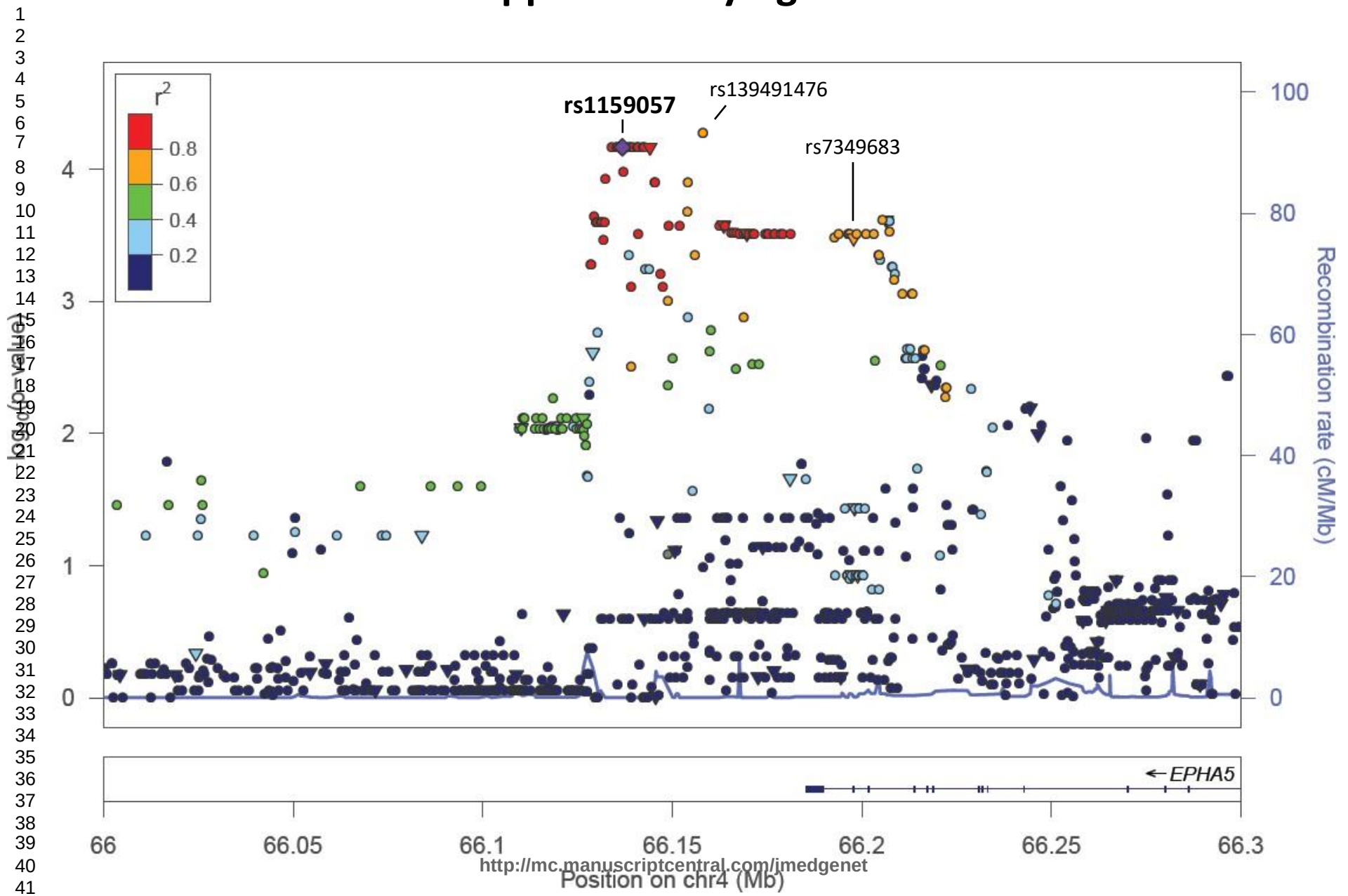
The neurologic assessment of the 23 Swedish patients, included a more extensive neurotoxicity assessment at cycle 3 or 4 and at the final cycle of chemotherapy. The evaluation consisted of 12 questions and 5 neurological tests according to Cassidy *et al.* and the severity of the toxicity resulted in a neurotoxicity score, Nscore (Cassidy *et al.*, Cancer Chemother Pharmacol, 1998, 41, 161–166). The patients were also asked to rate their inconveniences due to neurological adverse effects on a scale from 0 (no notice of neurological adverse effects) to 5 (unbearable). Both the patients' rating and Nscore at first response evaluation and at the final cycle were used for evaluation of the patient's individual neurotoxicity.

Supplementary Table 1. Meta-analysis of Baldwin et al top results.

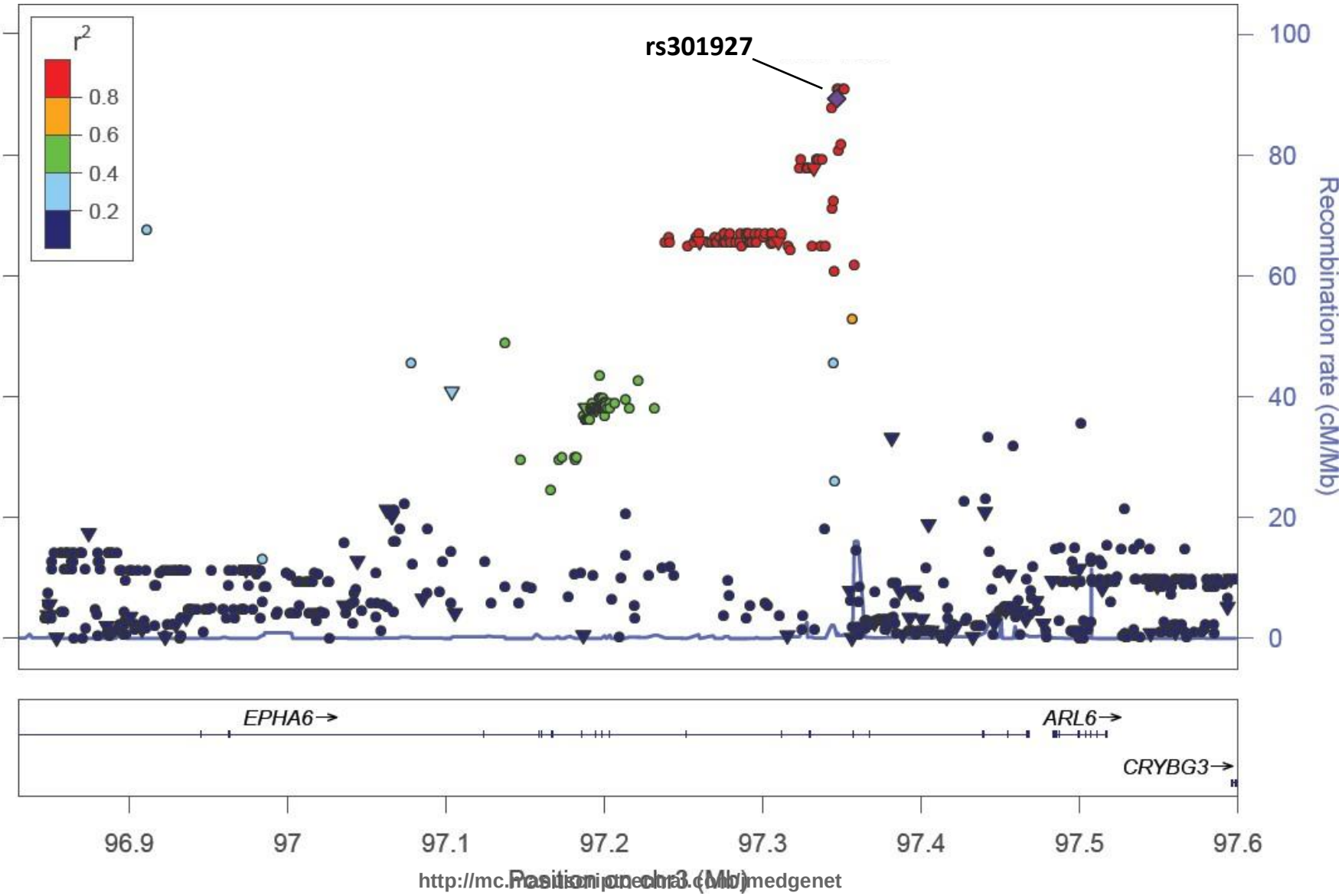
				Baldwin *et al.* Discovery (n=855)			Leandro-García *et al.* (n=143)			Meta-analysis	
SNP	Chr	Gene[a]	Alleles	MAF	HR (95% CI)	*P*	MAF	HR (95% CI)	*P*	HR (95% CI)	*P*
rs7349683	4	*EPHA5*	C/T	0.36	1.63 (1.34-1.98)	9.6×10^{-7}	0.40	1.83 (1.32-2.55)	3.33×10^{-4}	1.68 (1.42-1.99)	**1.44×10^{-9}**
rs4737264	8	*XKR4*	A/C	0.22	1.68 (1.36-2.09)	1.9×10^{-6}	0.23	1.79 (1.21-2.66)	3.48×10^{-3}	1.71 (1.41-2.06)	**3.11×10^{-8}**
rs10771973	12	*FGD4*	G/A	0.31	1.57 (1.30-1.91)	2.6×10^{-6}	0.33	1.35 (0.89-2.04)	0.1593	1.53 (1.28-1.82)	2.77×10^{-6}
rs16948748	17	*PITPNA*	T/G	0.04	2.37 (1.63-3.44)	2.7×10^{-6}	0.06	0.47 (0.17-1.31)	0.1508	2.34 (1.65-3.32)	1.95×10^{-6}
rs16916932	10	*CACNB2*	C/T	0.06	2.08 (1.51-2.87)	4.3×10^{-6}	0.06	0.79 (0.38-1.66)	0.5385	1.93 (1.44-2.58)	8.40×10^{-6}
rs17781082	12	*GRIP1/ CAND1*	C/T	0.42	1.60 (1.31-1.96)	4.3×10^{-6}	0.37	0.85 (0.58-1.25)	0.4160	1.50 (1.26-1.78)	5.52×10^{-6}
rs1903216	3	*BCL6/*	G/A	0.48	1.59 (1.30-1.95)	5.6×10^{-6}	0.48	1.00 (0.70-1.41)	0.9887	1.43 (1.20-1.69)	5.04×10^{-5}
rs2233335	8	*NDRG1*	T/G	0.38	0.65 (0.52-0.80)	5.2×10^{-5}	0.36	1.13 (0.80-1.61)	0.4884	0.76 (0.63-0.91)	2.81×10^{-3}

[a]Intergenic SNPs are denoted by the closest flanking annotated gene(s).

Supplementary figure 1



Supplementary figure 2



Supplementary figure 3