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Citation

Chhibber, A., J. Mefford, E. A. Stahl, S. A. Pendergrass, R. M. Baldwin, K. Owzar, M. Li, et al. 2014. "Polygenic Inheritance of Paclitaxel-Induced Sensory Peripheral Neuropathy Driven by Axon Outgrowth Gene Sets in CALGB 40101 (Alliance)." *The pharmacogenomics journal* 14 (4): 336-342. doi:10.1038/tpj.2014.2. <http://dx.doi.org/10.1038/tpj.2014.2>.

Published Version

doi:10.1038/tpj.2014.2

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Published in final edited form as:

Pharmacogenomics J. 2014 August ; 14(4): 336–342. doi:10.1038/tpj.2014.2.

Polygenic Inheritance of Paclitaxel-Induced Sensory Peripheral Neuropathy Driven by Axon Outgrowth Gene Sets in CALGB 40101 (Alliance)

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Conflict of Interest

The authors declare no conflict of interest.

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Abstract

Peripheral neuropathy is a common dose-limiting toxicity for patients treated with paclitaxel. For most individuals there are no known risk factors that predispose patients to the adverse event, and pathogenesis for paclitaxel-induced peripheral neuropathy is unknown. Determining whether there is a heritable component to paclitaxel induced peripheral neuropathy would be valuable in guiding clinical decisions and may provide insight into treatment of and mechanisms for the toxicity. Using genotype and patient information from the paclitaxel arm of CALGB 40101 (Alliance), a phase III clinical trial evaluating adjuvant therapies for breast cancer in women, we estimated the variance in maximum grade and dose at first instance of sensory peripheral neuropathy. Our results suggest that paclitaxel-induced neuropathy has a heritable component, driven in part by genes involved in axon outgrowth. Disruption of axon outgrowth may be one of the mechanisms by which paclitaxel treatment results in sensory peripheral neuropathy in susceptible patients.

Keywords

paclitaxel; neuropathy; polygenic; heritability; pathway

Introduction

Peripheral neuropathy is a common and often dose-limiting toxicity associated with cancer chemotherapy treatment. Paclitaxel is a chemotherapeutic agent in the taxane family, and functions by inhibiting microtubule assembly and inducing apoptosis. It is commonly prescribed in the treatment of carcinomas of the breast, ovary, lung, and head and neck¹. Sensory peripheral neuropathy induced by paclitaxel is dose-dependent and is the most common toxicity associated with this microtubule inhibitor. Severe toxicity (Grade 3 or higher) generally occurs in 5–10% of patients although rates as high as 30% have been reported for certain dosage regimens². Known risk factors for paclitaxel induced neuropathy include prior exposure to a neurotoxic agent or medical conditions associated with peripheral neuropathy, such as diabetes^{2–6}, though most patients who suffer from paclitaxel-induced neuropathy do not have an identifiable predisposition. The pathogenesis of paclitaxel induced peripheral neuropathy is unclear. Paclitaxel treatment may target axons, myelinating Schwann cells, or the dorsal root ganglion and neuron cell bodies of peripheral nerves⁷. At any of these sites, damage may be mediated by microtubule stabilization or mitochondrial disruption⁸. At very high single or cumulative doses almost all patients will experience some degree of peripheral neuropathy, but in certain susceptible patients neuropathy will occur at lower cumulative doses or with greater severity. Interindividual susceptibility to paclitaxel induced peripheral neuropathy may be driven by an overall increase in exposure to paclitaxel, or an increased sensitivity to damage or decreased capacity for repair at any of the putative targets of paclitaxel in the peripheral neuron.

Given the wide interindividual variability in incidence and severity of the toxicity independent of any known risk factors, it is likely that there is an underlying genetic basis

for susceptibility to paclitaxel-induced neuropathy. Small candidate gene studies focusing on genes involved in paclitaxel pharmacokinetics and pharmacodynamics (e.g., *ABCB1*, *CYP2C8*) or paclitaxel targets (e.g., β -tubulin) have had mixed results, with some identifying variants associated with neuropathy^{9–11}, and others failing to replicate previous results^{12, 13}. Recently, a genome-wide association study from this group¹⁴ identified several SNPs with moderate effect size in *FZD3*, *FGD4*, and *EPHA5* associated with severity or dose at onset of paclitaxel-induced sensory peripheral neuropathy. An independent genome-wide study identified SNPs in *RWDD3* and *TECTA* associated with onset of paclitaxel-induced neuropathy¹⁵, but these findings were not replicated by others¹⁶. The large number of putative causative variants identified, many with small effect size, and the discrepancies from study to study suggest a complex polygenic etiology for susceptibility to paclitaxel-induced neuropathy.

Pharmacogenomic studies, especially those involved in the study of drug toxicities, come with their own particular set of challenges. Sample sizes are often limited, and phenotype definitions can be imprecise¹⁷. This is compounded in cases where the toxicity does not appear to be driven by one or a few polymorphisms with large effect size, such as *CYP2D6* polymorphisms and morphine toxicity¹⁸, but rather by a number of variants each with small potential contribution to disease, as we propose is the case for paclitaxel-induced peripheral neuropathy. For these phenotypes, determining the extent to which genetic variability contributes to a particular toxicity can be challenging. Traditional heritability studies require large numbers of siblings or family structures that are not practicable, especially when studying potentially toxic drugs. Even when evidence for a heritable component to toxicity is available, candidate gene/candidate variant studies or traditional genome-wide association studies will likely be unable to identify variants with small effects that together explain a large portion of the expected heritability.

Recently, a method has been developed to estimate additive genetic variation or narrow-sense heritability driven by common SNPs (i.e. those typically captured on genotyping platforms) in unrelated individuals using linear mixed models^{19, 20}. This approach was applied to genome-wide SNP data in breast cancer patients treated with paclitaxel to determine the extent to which paclitaxel-induced sensory peripheral neuropathy is heritable and to identify causal SNPs driving this heritability.

Materials and Methods

Patient Data and Study Design

The patient cohort for this study was taken from the paclitaxel arm of CALGB 40101 (Alliance), a Phase III trial studying adjuvant therapy for patients with breast cancer; all patients in the current study were also enrolled in CALGB 60202 (Alliance), the pharmacogenomic companion study, and signed an IRB-approved, protocol-specific informed consent for use of their specimens. Paclitaxel was administered every two weeks over three hours at 175 mg/m² for four or six cycles. A total of 1,040 paclitaxel-treated individuals were included in the cohort; after quality control, including principal component analysis, call rate (>98%), and clustering performance, 859 Caucasian patients were retained for further analysis. Germline DNA was genotyped on the HumanHap610-Quad Genotyping

BeadChip (Illumina) platform. SNP quality control measures for minor allele frequency ($p < 0.01$), genotyping call rate ($> 99\%$), and Hardy-Weinberg equilibrium in controls (exact test $p < 0.001$) were applied using PLINK (v1.07). Genotyped data was imputed to call genotypes of un-typed SNPs using MACH^{21, 22} (1.0) and the 1000 Genomes²³ Pilot I (June 2010) data from unrelated Caucasian (CEU) individuals as a reference; imputed data was filtered for $r^2 > 0.9$. Recent publications describe further details regarding the pharmacogenomic¹⁴ and clinical²⁴ studies. Details regarding patient selection, SNP quality control and imputation are outlined in Supplemental Figure 1.

Phenotype

Two phenotypes are of interest in studying paclitaxel-induced neuropathy – severity of the neuropathy and cumulative dose at onset of neuropathy. These outcomes may be driven by distinct or overlapping sets of genes. Peripheral neuropathy was graded on a scale of 0 to 5 according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 2.0. The distribution of neuropathy grades in our cohort (Figure 1) matches expected numbers from prior clinical trials^{25, 26}. Because the linear mixed modeling approach requires a continuous quantitative or binary phenotype, both severity of neuropathy and dose at onset of neuropathy were treated as continuous variables. Severity of neuropathy was modeled using the highest grade of neuropathy over the course of treatment with log-transformed cumulative dose administered at highest grade of neuropathy (mg/m^2) as a covariate. For patients who did not experience the toxicity, cumulative dose administered over the course of the study was used as the covariate. Onset of neuropathy was modeled using deviance residuals from a time-to-event analysis as a continuous phenotype. The deviance residuals are a normalized transform of the martingale residuals, which estimate the difference at a particular cumulative dose t between observed (incidence of grade 2 or peripheral higher neuropathy, 0 or 1) and expected events (predicted hazard for neuropathy at dose t) for a given patient. Residuals from survival models have been previously used to model time to onset of various phenotypes as a quantitative trait when it is not possible to apply a survival model directly^{27–29}. The time-to-event analysis was conducted using a null Cox proportional hazards model without predictors, with time defined as cumulative paclitaxel dose and event defined as first instance of grade 2 or higher peripheral neuropathy¹⁴. For patients who did not experience grade 2 or higher neuropathy, cumulative dose administered over the course of the study was used, producing right-censored dosage date. Deviance residuals from the Cox score test were calculated using the survival package in R^{30, 31}.

Pathway Definitions

Pathways evaluated were selected based on putative pathology for paclitaxel-induced neuropathy. Five Gene Ontology³² (GO Release 2012-09-15) Biological Process terms were included: Axonogenesis (GO: 0007409), Myelination (GO: 0042552), Transmission of Nerve Impulse (GO: 0019226), Microtubule-Related Processes (GO: 0007017), and Mitochondrial Organization and Transport (GO: 0006839 and 0007005), along with a manually curated set of genes associated with congenital peripheral neuropathy³³ and a set of genes in the paclitaxel pharmacokinetic/pharmacodynamic pathway³⁴. For GO terms, all possible genes (regardless of evidence code) were included. For each pathway, gene

boundaries for the largest isoform of each gene were extracted from the UCSC Table Browser using UCSC gene annotations from human genome build 37 (hg19). These gene boundaries (plus an additional 10 kb upstream and downstream) were used to extract all dbSNP135³⁵ SNPs in the gene regions. Pathway SNP lists were used to extract the pathway-specific portion of the genome in PLINK (v1.07)³⁶.

For SNP sets grouped by position in the genome (genic vs. intergenic), gene and SNP annotations were extracted from the UCSC Table Browser using CCDS³⁷ gene annotations from human genome build 37 (hg19), and SNP annotations from dbSNP135. Genic regions were defined as 10 kb upstream and downstream of transcription start and stop sites. For genes with multiple CCDS isoforms, the longest isoform was used. The Biofilter³⁸ software (v2.0.0) was used to extract SNPs by genomic position.

Linear Mixed Modeling Heritability Analysis

Heritability estimates for the whole genome and for pathways were generated using the GCTA (v1.01) software tool³⁹. We estimated the genetic relatedness matrix (GRM) for 859 Caucasians using all post-QC genotyped SNPs. Principal components analysis was conducted using GCTA, and the first 20 eigenvectors for each individual were used as covariates in all subsequent analyses to control for any remaining population stratification. To ensure that all subjects in the study were unrelated, we excluded one of each of a pair of individuals with genetic relationship greater than 0.03, roughly corresponding to second cousins or closer familial relationships; ten individuals were excluded in this step. An additional four individuals were excluded due to incomplete phenotype information for a final population of 845 unrelated Caucasians (Supplemental Figure 1). All analyses were restricted to autosomes, and were conducted with the assumption that causal SNPs will have the same allele frequency distribution as genotyped SNPs.

For pathway specific heritability analyses, a separate GRM was constructed for each pathway and for its complement (whole genome GRM excluding SNPs in the pathway) using the set of 845 unrelated Caucasians. Total genetic variance for severity and onset of neuropathy was partitioned simultaneously onto pathway and “non-pathway” SNPs. Likewise, for genomic position based heritability analyses, total genetic variance for both phenotypes was partitioned onto genic and intergenic regions. To correct for the simultaneous evaluation of multiple pathways, GCTA p-values were Bonferroni corrected by multiplying each p-value by the number of pathways tested together (seven in the first round and ten in the second round). Empirical distributions representing the null hypothesis that the trait is not heritable were generated as follows for each pathway specific heritability estimate: for severity of neuropathy, residuals and expected values were extracted from linear regression of grade of neuropathy with log cumulative dose of paclitaxel and the first 20 principal components. For each of 1000 permutations, residuals were permuted, summed with expected values for each individual, and used to estimate pathway-specific heritability in GCTA. For onset of neuropathy, deviance residuals were calculated as described, then input as an independent variable in a linear regression including 20 principal components from which residuals and expected values were extracted. As with severity of neuropathy, for each of 1000 permutations, residuals were permuted, summed with expected values for

each individual, and used to estimate pathway-specific heritability in GCTA. Empirical p-values were generated by calculating the probability of obtaining a heritability estimate greater than that estimated from observed data.

PLINK Set Test

Briefly, the PLINK Set test as implemented in this study calculates the mean of all significant ($p < 0.05$) per-SNP p-values after filtering for SNPs in linkage disequilibrium ($r^2 > 0.5$). An empirical p-value is applied to each set test by permuting phenotype labels across individuals. SNP p-values for severity and onset of neuropathy were calculated in PLINK by linear regression of residuals from regression of grade of neuropathy on number of minor alleles, with log cumulative dose of paclitaxel and principal components as covariates in both initial regression and PLINK set test.

Results

The variance explained by common ($MAF > 1\%$) SNPs for paclitaxel-induced neuropathy was estimated in a cohort of 845 unrelated Caucasian breast cancer patients treated with single agent paclitaxel. Two outcomes were of interest – severity of neuropathy (measured on a grade of 0 to 5) and cumulative dose administered at onset of neuropathy (grade 2), both treated as continuous quantitative variables. The variance explained by all genotyped SNPs across the genome was estimated as 41% for severity of neuropathy and 55% for onset of neuropathy, but with high standard errors (44% and 47%, respectively) due to the small sample size. To narrow in on the causative SNPs driving heritability and reduce noise from non-causative SNPs, two methods were applied: (1) a genomic position based SNP selection, extracting SNPs in genic regions, and (2) a biological pathway based selection that extracted SNPs that fall in biological pathways that are associated with putative mechanisms for susceptibility to paclitaxel-induced neuropathy.

When partitioning the genome in SNP sets by genomic location (Figure 2), a trend toward higher heritability was found in genic regions for severity ($h^2 = 49\% \pm 37\%$, $p = 0.07$) and onset of peripheral neuropathy ($h^2 = 48\% \pm 35\%$, $p = 0.08$). For severity of peripheral neuropathy, pathway specific results show highest heritability estimates for the Axonogenesis gene set ($h^2 = 21\% \pm 12\%$, $p = 0.040$; Table 1). A complementary pathway analysis approach, the PLINK set test, was used to further extend our pathway based heritability results. Consistent with the GCTA analysis, only the Axonogenesis set is significant ($p = 0.012$) for severity of neuropathy using the set test (Supplemental Table 1). For onset of peripheral neuropathy, no significant signal of heritability was detected in any of the pathways tested (Supplemental Table 2).

“Children” of the GO Axonogenesis term, defined as terms with a “is_a” or “part_of” relationship with the Axonogenesis term, were subsequently tested for the severity of neuropathy phenotype (Table 2). Of the ten terms tested, GO Regulation of Axonogenesis (GO: 0050770), GO Axon Extension (GO: 0048675), and GO CNS Neuron Axonogenesis (GO: 0021955) showed strong heritability signals ($h^2 = 13\% \pm 6\%$ ($p = 0.009$), $10\% \pm 5\%$ ($p = 0.020$) and $5\% \pm 3\%$ ($p = 0.020$), respectively). To determine whether the signal from these three terms comes from independent genes in each set or overlapping genes in the

three sets, heritability estimates were calculated using the pair-wise and three-way union or intersection of the GO Regulation of Axonogenesis, GO Axon Extension, and GO CNS Neuron Axonogenesis sets. The union or intersection of the GO CNS Neuron Axonogenesis set with GO Axon Extension or GO Regulation of Axonogenesis sets resulted in lower heritability estimates than either independent set with high standard error (data not shown). For the GO Axon Extension and GO Regulation of Axonogenesis sets, the heritability signal from each independent set and the union and intersection sets are very similar (Figure 3), suggesting that a large portion of the SNPs driving the heritability in the Regulation and Extension sets come from the 44 genes found in both gene sets.

Heritability estimates were also calculated using imputed data; as with the genotyped SNPs, whole genome estimates of heritability with imputed SNPs had very high standard errors. For genomic position and pathway analyses, results from imputed data were similar to those described above for genotyped data, with a trend to higher heritability estimates in genic versus intergenic regions for the severity of peripheral neuropathy (Supplemental Table 3) and in the GO Axonogenesis set for severity of peripheral neuropathy (Supplemental Tables 4 – 6).

Discussion

These results suggest that a portion of variation in severity and onset of paclitaxel-induced sensory peripheral neuropathy is captured by additive effects of common SNPs in this clinical trial population. Previous studies have indicated that heritability is driven primarily by SNPs in genic regions⁴⁰, and a similar trend is found in our study. Within genic regions, we also noted a higher proportion of variance in severity and onset of peripheral neuropathy captured by SNPs in intronic regions (data not shown), but it is unclear whether this is due to a bias in the design of the genotyping chip or true bias in the genomic location of SNPs associated with paclitaxel induced neuropathy. If real, the enrichment of heritability signal in introns suggests that the majority of causal SNPs have subtle biological effects – for example, small changes in expression or stability that may be regulated by intronic SNPs, rather than overt changes in protein structure or function caused by variation in exons. This is consistent with a polygenic model in which many small, additive effects together contribute to the phenotype.

Further, a set of genes was identified that drive a substantial portion of the heritability of severity of paclitaxel-induced peripheral neuropathy, implicating axonogenesis, and more specifically the regulation of axon outgrowth, in the pathophysiology of this adverse event. These results are supported by evidence from human biopsies, electrophysiological studies, and animal and cell-based models that paclitaxel causes a distal axonopathy, in which the degeneration of axons occurs first at axon ends. This pattern of neuronal damage is consistent with a length-dependent neuropathy, targeting the long axons that extend into the hands and feet first, as typically occurs with paclitaxel induced neuropathy^{41–44}. Further, there is evidence that demyelination and ganglionopathy, if they do occur, are secondary to axon damage^{41, 44, 45}. The current results suggest that susceptibility to paclitaxel-induced neuropathy is caused in part by heightened sensitivity to or reduced capacity to repair this distal axon damage.

Of the 44 genes in the GO Axon Extension and GO Regulation of Axonogenesis overlap set (Supplemental Table 7), a number have been implicated in neuropathy, including hereditary neuropathy genes (*MAP1B*⁴⁶, *NGF*⁴⁷, *FXN*⁴⁸), genes with variants or expression signatures associated with diabetic or HIV-induced peripheral neuropathy (*APOE*^{49, 50}, *MAPT*⁵¹, *CDH4*⁵¹), genes involved in neurological pain pathways (*MT3*⁵², *TRPV2*⁵³, *CCR5*⁵⁴, *CXCL12*⁵⁵), and genes involved in response to or repair/prevention of peripheral nerve damage (*RYK*⁵⁶, *SLIT1*⁵⁷, *NTRK3*⁵⁸, *NGF*^{59, 60}, *TRPV2*⁵³, *NTN1*⁶¹, *NDEL1*⁶²). The majority (38) of these 44 genes fall in the GO term Regulation of Axon Extension (GO 0030516), which is a subset of both GO Regulation of Axonogenesis and GO Axon Extension.

The pathway results are also consistent with gene expression analyses in mouse and human studies of diabetic neuropathy. In a study examining the pathophysiology of diabetes-induced neuropathy the GO Axonogenesis term was identified as an overrepresented pathway in a differential expression analysis in the *db/db* vs *db/+* mouse sciatic nerve⁵¹. Similarly, the GO Regulation of Axonogenesis term was identified as an overrepresented set in genes up-regulated in sural nerve biopsies from patients with advanced progression of diabetic neuropathy⁶³. Although neuron damage is caused by different mechanisms in diabetes and following paclitaxel treatment, these results suggest that susceptibility to sensory peripheral neuropathy is driven by the same sets of genes.

Despite success in estimating heritability for paclitaxel-induced neuropathy and identifying a subset of the genome driving this heritability, some limitations in available methods and data are noted. One of the primary limitations of any pathway or gene set based analysis is the gene set definitions available. All available set definitions are limited by current knowledge about the pathway in question, and well curated sets are restricted to those pathways of interest to researchers. Further, the number of SNPs captured per gene varies, either because of true differences between number of variants or haplotype structure between genes, or because of differences in coverage between genes on the genotyping platform that was used. Such variability in local coverage is known to be a limitation in all commercial genotyping platforms⁶⁴. While imputation of missing SNPs did increase SNP density in each set, heritability estimates with imputed data were close to those with just genotyped data; because of the high imputation quality threshold used ($r^2 > 0.9$), it is likely that additional SNPs are in high LD with genotyped SNPs, adding little additional information. For onset of peripheral neuropathy, no significant signal of heritability was detected in any of the pathways tested, either because genes driving heritability of onset of neuropathy are in a pathway we did not select, or because the use of deviance residuals from the Cox proportional hazards regression rather than a direct proportional hazards regression did not adequately model the data. It is also possible that one or more of the selected pathways is incompletely annotated. Gene Ontology terms are annotated using a combination of experimental evidence and computational analyses, and can be both manually and electronically annotated^{32, 65}. The extensive set of sources for term annotation makes Gene Ontology the most comprehensive source of annotated terms available, but also contributes to significant noise (incorrectly assigned genes) being built into the terms. Unfortunately, highly accurate manually annotated gene sets are currently limited, and those that exist reflect the current body of knowledge regarding a given pathway. The Gene

Ontology was the only database that included gene sets for each of the peripheral neuropathy mechanisms of interest. For the GO set Axonogenesis, more restrictive set definitions were investigated, including limiting pathway genes to those annotated to Axonogenesis by experimental evidence and those that were direct associations. The GO Axonogenesis experimental set gave an estimate of heritability significantly lower than that derived from the complete gene set (8% vs 22% for the complete set), suggesting that using a more conservative gene annotation would result in loss of power (Supplemental Table 8).

The standard errors for the whole-genome heritability analyses are high due to the limited sample size. Large sample sizes are difficult to obtain in genomic studies of drug toxicities, since recruitment into these studies is often limited to existing clinical trials. However, by narrowing in on the “causative” SNPs, signals of heritability were obtained even with relatively small sample sizes. In this study, constraints were also imposed by the linear mixed modeling method applied, which requires a continuous or dichotomous phenotype. Although severity of neuropathy is best modeled as an ordinal variable, it is treated as a continuous quantitative variable for the purpose of this study. Likewise, onset of neuropathy is best fit in a survival model but deviance residuals from a survival model were used as a continuous trait in the current analysis. Despite these limitations, the results from the modified phenotype definitions are likely close to those that would be estimated from the application of non-linear phenotype definitions. For example, effect estimates for SNPs in biological pathways from severity of neuropathy modeled as a linear or ordinal variable (Supplemental Figure 3) or onset of neuropathy modeled as a linear phenotype or time-to-event analysis (Supplemental Figure 4) are highly correlated ($r^2 = 0.91$ and 0.97 , respectively). However it is important to note that, because of the constraints on the phenotype definition, we treat heritability estimates obtained from our analyses simply as an indication of association between a certain sets of SNPs and our phenotypes of interest, rather than absolute measures of percent of variance explained by a particular SNP set. Finally, a gene boundary cutoff of 10 kb was selected to ensure that the SNPs are associated with the genes in our pathway (as opposed to a neighboring gene), though at the cost of losing potential causative SNPs in upstream and downstream regulatory regions of a gene. Because most genetic variability appears to be explained by SNPs in or near genes⁴⁰ our approach likely captures a significant fraction of the variability explained by the genes in a given set.

In summary, these results suggest that there is a heritable component to the severity and dose to onset of paclitaxel-induced sensory peripheral neuropathy. Further, genes involved in axon outgrowth may modulate the severity of paclitaxel-induced neuropathy. Understanding the mechanisms and pathways involved in susceptibility to paclitaxel-induced sensory peripheral neuropathy will help identify therapies that can mitigate the toxicity and guide future drug development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The research for CALGB 60202 and 40101 was supported, in part, by grants from the National Cancer Institute (CA31946) to the Alliance for Clinical Trials in Oncology (Monica M. Bertagnolli, M.D., Chair) and to the Alliance Statistics and Data Center (Daniel J. Sargent, Ph.D., CA33601). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute.

This work was also supported in part by NIH grants U01 GM61390, U01 GM61393 and U01 HL065962 and the Biobank Japan Project funded by the Japanese Ministry of Education, Culture, Sports, Science and Technology. The genotyping used for this work was generated as part of the NIH Pharmacogenomics Research Network-RIKEN Center for Genomic Medicine Global Alliance. Aparna Chhibber and Megan Li were supported in part by NIH Training Grant T32 GM007175.

References

1. Rowinsky EK, Donehower RC. Paclitaxel (taxol). *N Engl J Med*. 1995; 332:1004–1014. [PubMed: 7885406]
2. Lee JJ, Swain SM. Peripheral neuropathy induced by microtubule-stabilizing agents. *J Clin Oncol*. 2006; 24:1633–1642. [PubMed: 16575015]
3. Donehower RC, Rowinsky EK, Grochow LB, Longnecker SM, Ettinger DS. Phase I trial of taxol in patients with advanced cancer. *Cancer Treat Rep*. 1987; 71:1171–1177. [PubMed: 2891441]
4. Postma TJ, Vermorken JB, Liefing AJ, Pinedo HM, Heimans JJ. Paclitaxel-induced neuropathy. *Ann Oncol*. 1995; 6:489–494. [PubMed: 7669713]
5. Rowinsky EK, Chaudhry V, Cornblath DR, Donehower RC. Neurotoxicity of taxol. *J Natl Cancer Inst Monogr*. 1993:107–115. [PubMed: 7912516]
6. Rowinsky EK, Eisenhauer EA, Chaudhry V, Arbuck SG, Donehower RC. Clinical toxicities encountered with paclitaxel (Taxol). *Semin Oncol*. 1993; 20:1–15. [PubMed: 8102012]
7. Lipton RB, Apfel SC, Dutcher JP, Rosenberg R, Kaplan J, Berger A, et al. Taxol produces a predominantly sensory neuropathy. *Neurology*. 1989; 39:368–373. [PubMed: 2564647]
8. Flatters SJ, Bennett GJ. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: evidence for mitochondrial dysfunction. *Pain*. 2006; 122:245–257. [PubMed: 16530964]
9. Leandro-Garcia LJ, Leskela S, Jara C, Green H, Avall-Lundqvist E, Wheeler HE, et al. Regulatory polymorphisms in β -tubulin IIa are associated with paclitaxel-induced peripheral neuropathy. *Clin Cancer Res*. 2012; 18:4441–4448. [PubMed: 22718863]
10. Leskela S, Jara C, Leandro-Garcia LJ, Martinez A, Garcia-Donas J, Hernando S, et al. Polymorphisms in cytochromes P450 2C8 and 3A5 are associated with paclitaxel neurotoxicity. *Pharmacogenomics J*. 2011; 11:121–129. [PubMed: 20212519]
11. Sissung TM, Mross K, Steinberg SM, Behringer D, Figg WD, Sparreboom A, et al. Association of *ABCB1* genotypes with paclitaxel-mediated peripheral neuropathy and neutropenia. *Eur J Cancer*. 2006; 42:2893–2896. [PubMed: 16950614]
12. Bergmann TK, Green H, Brasch-Andersen C, Mirza MR, Herrstedt J, Holund B, et al. Retrospective study of the impact of pharmacogenetic variants on paclitaxel toxicity and survival in patients with ovarian cancer. *Eur J Clin Pharmacol*. 2011; 67:693–700. [PubMed: 21327421]
13. Ofverholm A, Einbeigi Z, Manouchehrpour S, Albertsson P, Skrtic S, Enerback C. The *ABCB1* 3435 T allele does not increase the risk of paclitaxel-induced neurotoxicity. *Oncol Lett*. 2010; 1:151–154. [PubMed: 22966274]
14. Baldwin RM, Owzar K, Zembutsu H, Chhibber A, Kubo M, Jiang C, et al. A genome-wide association study identifies novel loci for paclitaxel-induced sensory peripheral neuropathy in CALGB 40101. *Clin Cancer Res*. 2012; 18:5099–5109. [PubMed: 22843789]
15. Schneider BP, Li L, Miller K, Flockhart DA, Radovich M, Hancock BA, et al. Genetic associations with taxane-induced neuropathy by a genome-wide association study in E5103. *J Clin Oncol*. 2011; 29(Suppl):1000.

16. Bergmann TK, Vach W, Feddersen S, Eckhoff L, Green H, Herrstedt J, et al. GWAS-based association between *RWDD3* and *TECTA* variants and paclitaxel induced neuropathy could not be confirmed in Scandinavian ovarian cancer patients. *Acta Oncol.* 2013; 52:871–874. [PubMed: 22877241]
17. Motsinger-Reif AA, Jorgenson E, Relling MV, Kroetz DL, Weinshilboum R, Cox NJ, et al. Genome-wide association studies in pharmacogenomics: successes and lessons. *Pharmacogenet Genomics.* 2010; 23:389–394.
18. Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder SJ. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. *Lancet.* 2006; 368:704. [PubMed: 16920476]
19. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am J Hum Genet.* 2011; 88:294–305. [PubMed: 21376301]
20. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet.* 2010; 42:565–569. [PubMed: 20562875]
21. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet.* 2009; 10:387–406. [PubMed: 19715440]
22. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol.* 2010; 34:816–834. [PubMed: 21058334]
23. Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, et al. A map of human genome variation from population-scale sequencing. *Nature.* 2010; 467:1061–1073. [PubMed: 20981092]
24. Shulman LN, Cirrincione CT, Berry DA, Becker HP, Perez EA, O'Regan RO, et al. Six cycles of doxorubicin and cyclophosphamide or paclitaxel are not superior to 4 cycles as adjuvant chemotherapy for breast cancer in women with 0–3 positive axillary nodes: CALGB 40101. *J Clin Oncol.* 2012; 30:4071–4076. [PubMed: 22826271]
25. Muggia FM, Braly PS, Brady MF, Sutton G, Niemann TH, Lentz SL, et al. Phase III randomized study of cisplatin versus paclitaxel versus cisplatin and paclitaxel in patients with suboptimal stage III or IV ovarian cancer: a gynecologic oncology group study. *J Clin Oncol.* 2000; 18:106–115. [PubMed: 10623700]
26. Perez EA, Vogel CL, Irwin DH, Kirshner JJ, Patel R. Multicenter phase II trial of weekly paclitaxel in women with metastatic breast cancer. *J Clin Oncol.* 2001; 19:4216–4223. [PubMed: 11709565]
27. Bouzigon E, Ulgen A, Dizier MH, Siroux V, Lathrop M, Kauffmann F, et al. Evidence for a pleiotropic QTL on chromosome 5q13 influencing both time to asthma onset and asthma score in French EGEA families. *Hum Genet.* 2007; 121:711–719. [PubMed: 17473937]
28. Dickson MR, Li J, Wiener HW, Perry RT, Blacker D, Bassett SS, et al. A genomic scan for age at onset of Alzheimer's disease in 437 families from the NIMH Genetic Initiative. *Am J Med Genet B Neuropsychiatr Genet.* 2008; 147B:784–792. [PubMed: 18189239]
29. Hunt KJ, Lehman DM, Arya R, Fowler S, Leach RJ, Goring HH, et al. Genome-wide linkage analyses of type 2 diabetes in Mexican Americans: the San Antonio Family Diabetes/Gallbladder Study. *Diabetes.* 2005; 54:2655–2662. [PubMed: 16123354]
30. Therneau TM. A package for survival analysis in S. 2012
31. Therneau, TM.; Grambsch, PM. Modeling Survival Data: Extending the Cox Model. Springer-Verlag: New York; 2000.
32. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.* 2000; 25:25–29. [PubMed: 10802651]
33. Timmerman, V. Inherited peripheral neuropathies Mutation database. 2011. <http://www.molgen.ua.ac.be/cmtmutations/Home/Default.cfm>
34. Oshiro C, Marsh S, McLeod H, Carrillo M, Klein T, Altman R. Taxane Pathway. *Pharmacogenet Genomics.* 2009; 19:979–983. [PubMed: 21151855]
35. Smigielski EM, Sirotkin K, Ward M, Sherry ST. dbSNP: a database of single nucleotide polymorphisms. *Nucleic Acids Res.* 2000; 28:352–355. [PubMed: 10592272]

36. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007; 81:559–575. [PubMed: 17701901]
37. Pruitt KD, Harrow J, Harte RA, Wallin C, Diekhans M, Maglott DR, et al. The consensus coding sequence (CCDS) project: Identifying a common protein-coding gene set for the human and mouse genomes. *Genome Res.* 2009; 19:1316–1323. [PubMed: 19498102]
38. Bush WS, Dudek SM, Ritchie MD. Biofilter: a knowledge-integration system for the multi-locus analysis of genome-wide association studies. *Pac Symp Biocomput.* 2009:368–379. [PubMed: 19209715]
39. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet.* 2011; 88:76–82. [PubMed: 21167468]
40. Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM, et al. Genome partitioning of genetic variation for complex traits using common SNPs. *Nat Genet.* 2011; 43:519–525. [PubMed: 21552263]
41. Chaudhry V, Rowinsky EK, Sartorius SE, Donehower RC, Cornblath DR. Peripheral neuropathy from taxol and cisplatin combination chemotherapy: clinical and electrophysiological studies. *Ann Neurol.* 1994; 35:304–311. [PubMed: 7907208]
42. Letourneau PC, Ressler AH. Inhibition of neurite initiation and growth by taxol. *J Cell Biol.* 1984; 98:1355–1362. [PubMed: 6143759]
43. Melli G, Jack C, Lambrinos GL, Ringkamp M, Hoke A. Erythropoietin protects sensory axons against paclitaxel-induced distal degeneration. *Neurobiol Dis.* 2006; 24:525–530. [PubMed: 17010629]
44. Sahenk Z, Barohn R, New P, Mendell JR. Taxol neuropathy. Electrodiagnostic and sural nerve biopsy findings. *Arch Neurol.* 1994; 51:726–729. [PubMed: 7912506]
45. Roytta M, Raine CS. Taxol-induced neuropathy: chronic effects of local injection. *J Neurocytol.* 1986; 15:483–496. [PubMed: 2427662]
46. Allen E, Ding J, Wang W, Pramanik S, Chou J, Yau V, et al. Gigaxonin-controlled degradation of MAP1B light chain is critical to neuronal survival. *Nature.* 2005; 438:224–228. [PubMed: 16227972]
47. Einarsdottir E, Carlsson A, Minde J, Toolanen G, Svensson O, Solders G, et al. A mutation in the nerve growth factor beta gene (*NGFB*) causes loss of pain perception. *Hum Mol Genet.* 2004; 13:799–805. [PubMed: 14976160]
48. Becker E, Richardson DR. Frataxin: its role in iron metabolism and the pathogenesis of Friedreich's ataxia. *Int J Biochem Cell Biol.* 2001; 33:1–10. [PubMed: 11167127]
49. Bedlack RS, Edelman D, Gibbs JW 3rd, Kelling D, Strittmatter W, Saunders AM, et al. *APOE* genotype is a risk factor for neuropathy severity in diabetic patients. *Neurology.* 2003; 60:1022–1024. [PubMed: 12654974]
50. Corder EH, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkins J, et al. HIV-infected subjects with the E4 allele for *APOE* have excess dementia and peripheral neuropathy. *Nat Med.* 1998; 4:1182–1184. [PubMed: 9771753]
51. Pande M, Hur J, Hong Y, Backus C, Hayes JM, Oh SS, et al. Transcriptional profiling of diabetic neuropathy in the BKS *db/db* mouse: a model of type 2 diabetes. *Diabetes.* 2011; 60:1981–1989. [PubMed: 21617178]
52. Zurowski D, Nowak L, Machowska A, Wordliczek J, Thor PJ. Exogenous melatonin abolishes mechanical allodynia but not thermal hyperalgesia in neuropathic pain. The role of the opioid system and benzodiazepine-gabaergic mechanism. *J Physiol Pharmacol.* 2012; 63:641–647. [PubMed: 23388480]
53. Hori K, Ozaki N, Suzuki S, Sugiura Y. Upregulations of P2X(3) and ASIC3 involve in hyperalgesia induced by cisplatin administration in rats. *Pain.* 2010; 149:393–405. [PubMed: 20378247]
54. Padi SS, Shi XQ, Zhao YQ, Ruff MR, Baichoo N, Pert CB, et al. Attenuation of rodent neuropathic pain by an orally active peptide, RAP-103, which potently blocks CCR2- and CCR5-mediated monocyte chemotaxis and inflammation. *Pain.* 2012; 153:95–106. [PubMed: 22033364]

55. Benamar K, Geller EB, Adler MW. First in vivo evidence for a functional interaction between chemokine and cannabinoid systems in the brain. *J Pharmacol Exp Ther.* 2008; 325:641–645. [PubMed: 18281594]
56. Li X, Li YH, Yu S, Liu Y. Upregulation of Ryk expression in rat dorsal root ganglia after peripheral nerve injury. *Brain Res Bull.* 2008; 77:178–184. [PubMed: 18773946]
57. Yi XN, Zheng LF, Zhang JW, Zhang LZ, Xu YZ, Luo G, et al. Dynamic changes in Robo2 and Slit1 expression in adult rat dorsal root ganglion and sciatic nerve after peripheral and central axonal injury. *Neurosci Res.* 2006; 56:314–321. [PubMed: 16979769]
58. Gao WQ, Dybdal N, Shinsky N, Murnane A, Schmelzer C, Siegel M, et al. Neurotrophin-3 reverses experimental cisplatin-induced peripheral sensory neuropathy. *Ann Neurol.* 1995; 38:30–37. [PubMed: 7611721]
59. Apfel SC, Lipton RB, Arezzo JC, Kessler JA. Nerve growth factor prevents toxic neuropathy in mice. *Ann Neurol.* 1991; 29:87–90. [PubMed: 1705109]
60. Peterson ER, Crain SM. Nerve growth factor attenuates neurotoxic effects of taxol on spinal cord-ganglion explants from fetal mice. *Science.* 1982; 217:377–379. [PubMed: 6124041]
61. Wilson BD, Ii M, Park KW, Suli A, Sorensen LK, Larrieu-Lahargue F, et al. Netrins promote developmental and therapeutic angiogenesis. *Science.* 2006; 313:640–644. [PubMed: 16809490]
62. Toth C, Shim SY, Wang J, Jiang Y, Neumayer G, Belzil C, et al. Ndel1 promotes axon regeneration via intermediate filaments. *PLoS One.* 2008; 3:e2014. [PubMed: 18431495]
63. Hur J, Sullivan KA, Pande M, Hong Y, Sima AA, Jagadish HV, et al. The identification of gene expression profiles associated with progression of human diabetic neuropathy. *Brain.* 2011; 134:3222–3235. [PubMed: 21926103]
64. Lindquist KJ, Jorgenson E, Hoffmann TJ, Witte JS. The impact of improved microarray coverage and larger sample sizes on future genome-wide association studies. *Genet Epidemiol.* 2013; 37:383–392. [PubMed: 23529720]
65. Harris MA, Clark J, Ireland A, Lomax J, Ashburner M, Foulger R, et al. The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Res.* 2004; 32:D258–D261. [PubMed: 14681407]

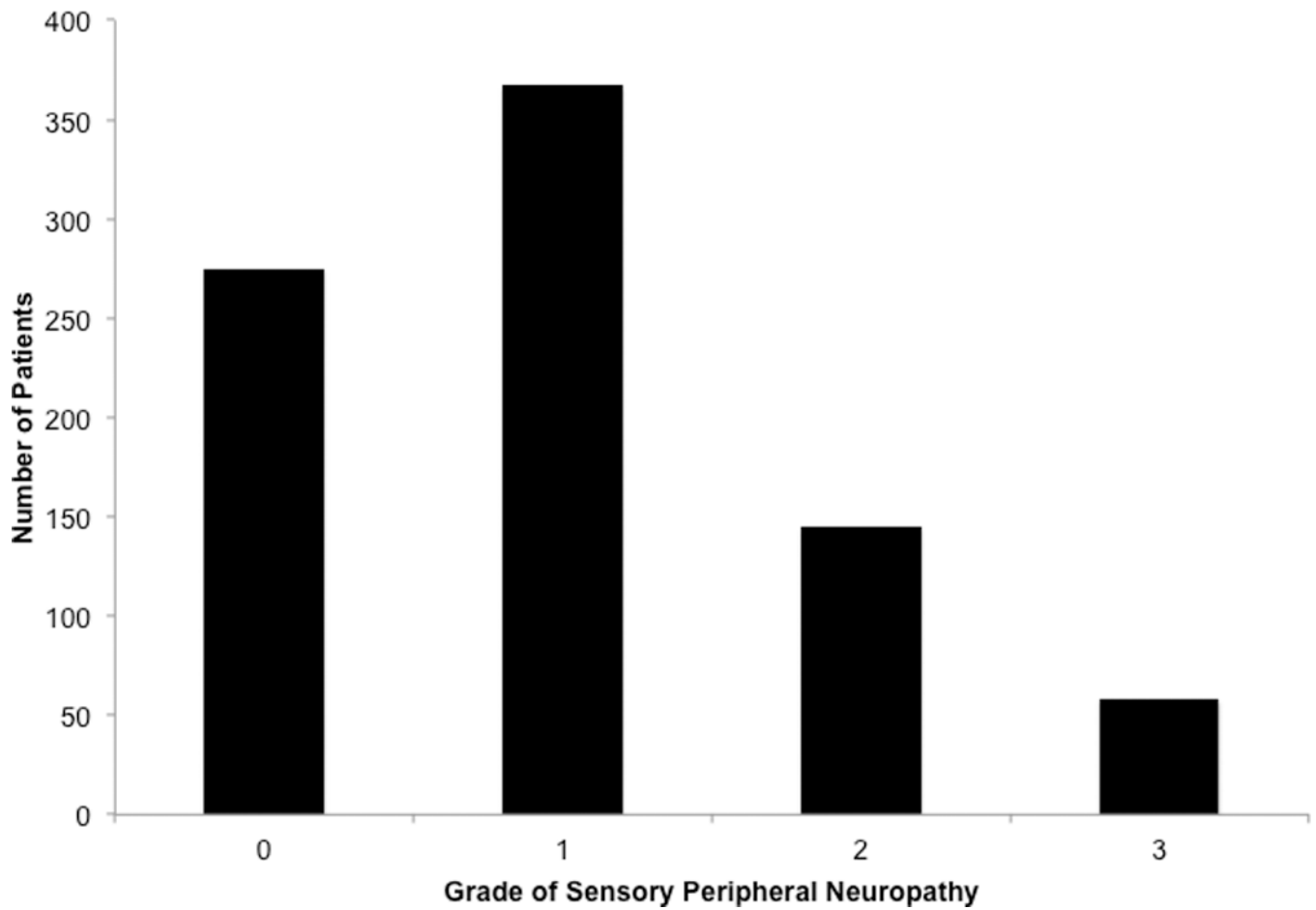


Figure 1. Distribution of sensory peripheral neuropathy in the study population

The distribution of the highest reported grade of sensory peripheral neuropathy is shown for 849 unrelated genetic Europeans from the paclitaxel arm of CALGB 40101. Toxicity is measured using the NCI-CTCAE Scale v2.

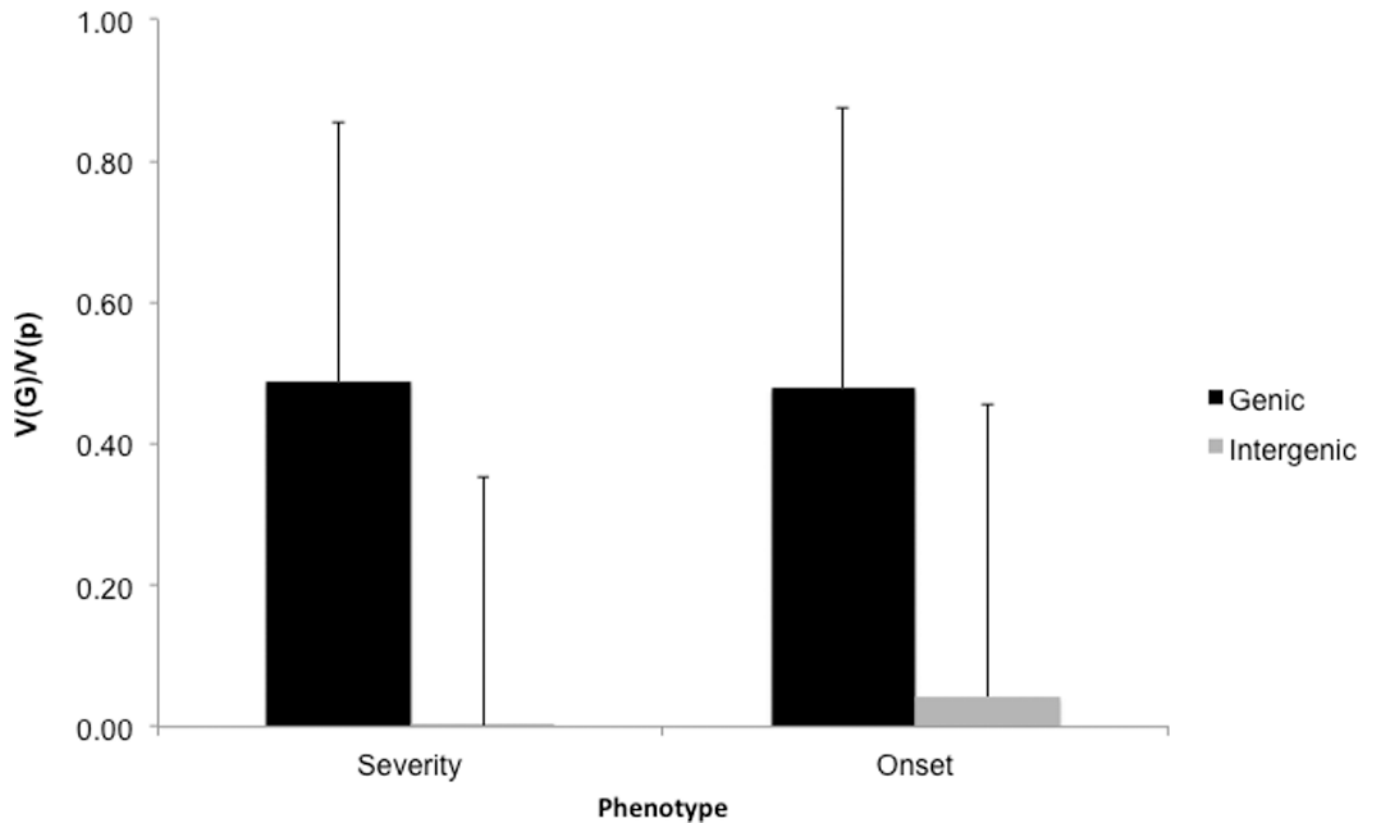


Figure 2. Heritability estimates for severity and onset of paclitaxel-induced sensory peripheral neuropathy for SNPs in genic and intergenic regions
Total genomic variance for both severity and onset of neuropathy was partitioned onto genic and intergenic regions. The error bars denote the SE for the heritability estimates.

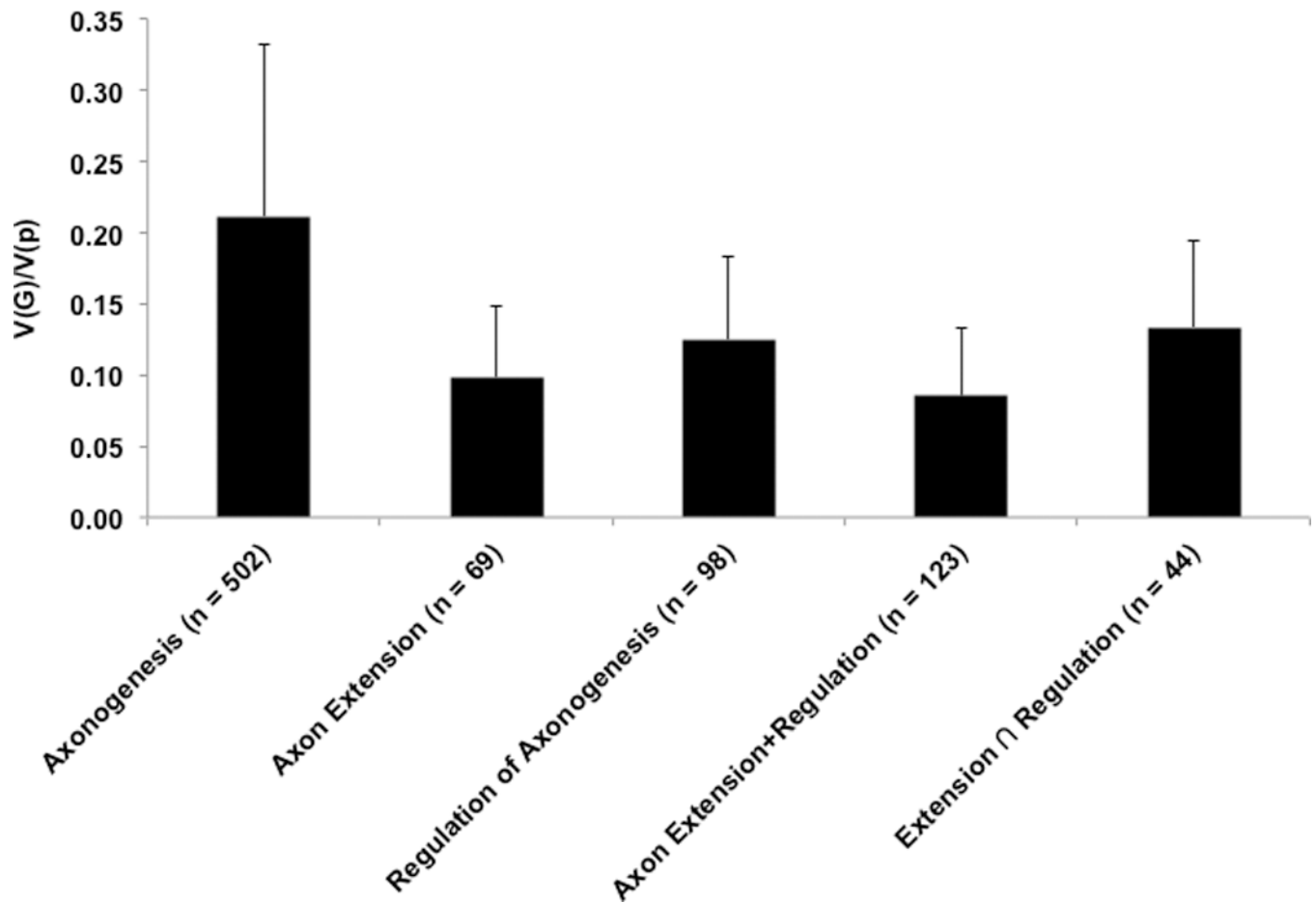


Figure 3. Heritability estimates for severity of paclitaxel-induced sensory peripheral neuropathy for SNPs in selected GO biological pathways

Heritability was estimated for sets of SNPs within all pathways contained within the GO Axonogenesis pathway. Results are shown (heritability \pm SE) for those pathways with significant ($P < 0.05$) heritability signals. The heritability estimates for the intersection between and union of the Axon Extension and Regulation of Axonogenesis are also shown.

Table 1

Heritability estimates for severity of paclitaxel-induced sensory neuropathy using SNPs in biological pathways implicated in the toxicity

Pathway	Heritability Estimates				Pathway Characteristics			
	V(G)/V(p) ¹	SE	p ²	Padj ³	Empirical p ⁴	# Genes	Size (Mb)	#SNPs
GO Axonogenesis	0.213	0.120	0.040	0.28	0.011	502	78.0	17,581
GO Impulse Transmission	0.000	0.122	0.500	1	0.999	746	106	22,886
GO Myelination	0.029	0.035	0.200	1	0.255	75	6.86	1,336
Congenital Peripheral Neuropathy	0.000	0.030	0.500	1	0.999	40	4.03	947
Paclitaxel Pharmacokinetics/ Pharmacodynamics	0.011	0.017	0.300	1	0.221	10	1.20	402
GO Mitochondrial Transport and Organization	0.012	0.055	0.400	1	0.545	274	19.7	3,668
GO Microtubule Related Processes	0.000	0.072	0.500	1	0.999	34	3.55	5,775

¹ Heritability was estimated for sets of SNPs within ± 10 kb of genes in biological pathways implicated in the pathophysiology of paclitaxel-induced sensory peripheral neuropathy. The congenital neuropathy and paclitaxel pharmacokinetics/pharmacodynamics pathways were manually constructed from the literature.

² P-value from GCTA. Software upper limit for p-value is 0.5; maximal values are noted as 1.

³ P-value corrected for seven observations.

⁴ P-value from permutation analysis.

Table 2

Heritability estimates for severity of neuropathy captured by SNPs in subsets of the GO Axonogenesis set

GO Axonogenesis Children	Heritability Estimates					Pathway Characteristics			
	V(G)/V(p) ¹	SE	p ²	Padj ³	Empirical p ⁴	# Genes	Size (Mb)	#SNPs	
Axonal Fasciculation	0.000	0.025	0.5	1	0.999	15	2.89	922	
Peripheral Neuron Axonogenesis	0.005	0.010	0.3	1	0.203	2	0.13	15	
Axon Guidance	0.000	0.019	0.5	1	0.999	362	57.51	699	
Axonogenesis in Innervation	0.011	0.015	0.2	1	0.146	3	0.15	19	
Axon Regeneration	0.000	0.013	0.5	1	0.999	29	3.31	314	
CNS Neuron Axonogenesis	0.051	0.031	0.020	0.2	0.028	26	6.32	935	
Axon Extension	0.097	0.050	0.020	0.2	0.003	70	8.88	1,862	
Regulation of Axonogenesis	0.130	0.059	0.009	0.09	0.001	104	20.85	3,239	
Collateral Sprouting	0.012	0.019	0.3	1	0.26	13	3.10	396	
Axon Target Recognition	0.000	0.010	0.5	1	0.999	4	0.27	34	

¹ Heritability was estimated for sets of SNPs within ± 10 kb of genes in children (subsets) of the GO Axonogenesis set.

² P-value from GCTA. Software upper limit for p-value is 0.5; maximal values are noted as 1.

³ P-value corrected for ten observations.

⁴ P-value from permutation analysis.