Clinical and Genome Wide Analysis of Cisplatin-Induced Peripheral Neuropathy in Survivors of Adult-Onset Cancer

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Abstract

Purpose—Our purpose was to characterize the clinical influences, genetic risk factors, and gene mechanisms contributing to persistent cisplatin-induced peripheral neuropathy (CisIPN) in testicular cancer survivors (TCS).

Experimental Design—TCS given cisplatin-based therapy completed the validated EORTC QLQ-CIPN20 questionnaire. An ordinal CisIPN phenotype was derived and associations with age, smoking, excess drinking, hypertension, body mass index, diabetes, hypercholesterolemia, cumulative cisplatin dose, and self-reported health were examined for 680 TCS. Genotyping was performed on the Illumina HumanOmniExpressExome chip. Following quality control and

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imputation, 5.1 million SNPs in 680 genetically European TCS formed the input set. GWAS and PrediXcan were used to identify genetic variation and genetically-determined gene expression traits, respectively, contributing to CisIPN. We evaluated two independent datasets for replication: Vanderbilt's electronic health database, BioVU and CALGB 90401 trial.

Results—Eight sensory items formed a subscale with good internal consistency (Cronbach α=0.88). Variables significantly associated with CisIPN included age at diagnosis (OR per year=1.06, p=2×10^{-9}), smoking (OR=1.54, p=0.004), excess drinking (OR=1.83, p=0.007), and hypertension (OR=1.61, p=0.03). CisIPN was correlated with lower self-reported health (OR=0.56; p=2.6×10^{-9}) and weight gain adjusted for years since treatment (OR per kg/m^2=1.05, p=0.004). PrediXcan identified lower expressions of *MIDN* and *RPRD1B,* and higher *THEM5* expression as associated with CisIPN (p-value for each $< 5 \times 10^{-6}$) with replication of *RPRD1B* meeting significance criteria (Fisher's combined p=0.0089).

Conclusions—CisIPN is associated with age, modifiable risk factors and genetically-determined expression level of *RPRD1B*. Further study of implicated genes could elucidate the pathophysiologic underpinnings of CisIPN.

Keywords

cisplatin; peripheral neuropathy; genome wide association study; cancer survivorship; Platinum Study; young adult; testicular cancer; germ cell tumor; patient reported outcomes; pharmacogenetics

INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most common and often debilitating adverse effects of modern chemotherapy (1, 2). Platinum analogs, taxanes, vinca alkaloids, thalidomide, and epothilones are associated with CIPN, although with different clinical manifestations, which include sensory, motor and autonomic symptoms (1). Mechanisms underlying CIPN remain largely unclear (2). Unlike taxane-related CIPN, which can be reversible (2), cisplatin-induced peripheral neuropathy (CisIPN) can be cumulative and progressive and cause long-term effects on overall quality of life (QoL) and physical function (3). Studies have suggested that age, race, diabetes and obesity may be risk factors for CIPN (4, 5), but results have been conflicting (6–11).

No agents are currently available to prevent or treat CIPN (12). Management of CIPN is complicated by the lack of reliable means to identify at-risk patients. While genetic variants could address unexplained phenotypic variability, heterogeneity of treatment regimens and the lack of long-term patient follow-up complicate pharmacogenetic studies of chemotherapeutic toxicities, including CIPN. However, cisplatin-based chemotherapy for testicular cancer is relatively homogeneous, and is curative (13). Testicular cancer survivors (TCS) are typically young at diagnosis, and the period of survivorship can span upwards of 50 years, providing an ideal model to investigate long-term adverse effects of cisplatin-based chemotherapy. These include hearing impairment, tinnitus, renal dysfunction, cardiovascular disease, secondary malignancies, CisIPN, and others (14). CisIPN most commonly presents

as a sensory neuropathy in a stocking-glove distribution with numbness, tingling, paresthesias, loss of proprioception, hyperalgesia and/or allodynia (3).

Although cisplatin is one of the most widely prescribed chemotherapeutics, there are few data available on genetic variants associated with CisIPN. Prior investigations assessed variants in a few candidate genes in small numbers of patients (range 104–238), who often also received other neurotoxic agents (15–18). In this study, we used the extensively validated EORTC-CIPN20 scale (19) and other patient-reported outcomes (PRO) to characterize CisIPN in a large number of TCS, the impact on self-reported health, and associated clinical and demographic variables. We additionally conducted, to our knowledge, the first genome-wide association study of CisIPN.

PATIENTS AND METHODS

Patients

All patients were enrolled in The Platinum Study, a cross sectional study that includes 8 cancer centers in the United States and Canada (14). Eligibility criteria included: men diagnosed with a histologically or serologically confirmed germ cell tumor (GCT), age <55 years at diagnosis and >18 years at enrollment, treatment with cisplatin-based chemotherapy for either initial GCT or recurrence, no antecedent chemotherapy for another primary cancer and no subsequent salvage chemotherapy. Of all eligible patients, 93% agreed to participate. Study procedures were approved by the Human Subjects Review Board at each institution. All patients provided written consent for participation in study procedures, including genetic analyses.

Data Collection

TCS visiting the clinic for follow-up and who provided written consent for participation underwent a brief physical examination, including measurement of height and weight. Body mass index (BMI) (kg/m^2) was defined as normal, overweight, obese, or morbidly obese (<25, 25 to <30, 30 to <40, and 40 kg/m^2, respectively). Figure S1 illustrates the data collection pipeline. Patients completed questionnaires with regard to neurotoxic symptoms, comorbidities, lifestyle behaviors, perceived health and medication use at the time of enrollment (range: 1–30 years after treatment completion). These included the EORTC-QLQ-CIPN20 questionnaire with questions listed in Table S1 (20). Data abstracted from medical records with standardized forms included all variables related to GCT diagnosis and treatment. For each administered cytotoxic drug, information was collected on name, dose, date(s) of administration, number of cycles, and cumulative dose.

Phenotype Analysis

We evaluated the frequency of sensory neuropathy using 9 items in the EORTC-CIPN20 (19). We assessed inter-item associations as well as relationships with motor and autonomic neuropathy by variance analysis using principal component analysis (PCA), following conversion of the Likert scale to a 0–3 numeric scale: 0 for "none", 1 for "a little", 2 for "quite a bit", 3 for "very much". We evaluated subscale internal consistency using Cronbach α (21). Following exclusion of the hearing impairment item, we created a summary statistic

of CisIPN mathematically equivalent to the standard scoring algorithm (22): rather than summing item scores and scaling to 0–100, we took the mean of the scores, effectively summing scores and scaling to 0–3 and imputing missing data points from available ones (11 samples were missing one response, and two samples were missing three responses). The ceiling function was then used to place means into four ordinal groups reflecting the average severity across symptoms created as follows: none (0; mean = 0), a little (1; 0 < mean ≤ 1), quite a bit (2; 1 < mean ≤ 2), very much (3; 2 < mean ≤ 3). Groups 2 and 3 were combined due to low frequency.

Ordinal Regressions with Relevant Variables

We determined the statistical association between CisIPN and data abstracted from the medical records at the time of treatment and data collected at the time of clinical evaluation. Dependent variables abstracted from the medical records included GCT treatment regimen, cumulative cisplatin dose, age and BMI at the initiation of treatment. Data collected at clinical evaluation included age and BMI, self-reported smoking status, alcohol consumption, blood pressure, diabetes, and hypercholesterolemia. Age was analyzed as a continuous variable. Ever smokers were defined as those responding affirmatively to "have you ever smoked?". Excessive drinkers were defined as those who reported consuming ≥ 2 drinks/day on average in the past year. Hypertensive patients were those who both indicated that they had received a diagnosis of high blood pressure <u>and</u> were taking prescription medication for it at the time of evaluation. Diabetic patients were those who indicated they had 1) diabetes requiring insulin or 2) diabetes requiring tablets or pills. We also took into consideration those patients currently taking prescription medication for high cholesterol in view of the small risk of neuropathy associated with statin therapy (23). TCS were also asked to rate their health as "excellent," "very good," "good," "fair," or "poor", and to report the average time per week engaged in various physical activities during the past year (24). Total kilocalories per week were estimated by summing Metabolic Equivalent of Task (MET)-hours for each physical activity and multiplying by the participant's weight (kilograms). Kilocalories per week were calculated and categorized as vigorous (≥ 6 METs), moderate (3 to <6 METs), and less than moderate (<3 METs) physical activity (25). Weight gain was determined as the difference in BMI between initiation of chemotherapy and study evaluation (Δ kg/m^2). Association with the CisIPN phenotype was evaluated in proportional odds ordered logistic regression models at $\alpha = 0.05$. All univariate regressions were adjusted for age at diagnosis (covariate). A multi-variable regression model was constructed by using all significant variables in the age-adjusted univariate analysis.

Genotyping and Imputation

DNA extraction was performed from peripheral blood. SNPs were genotyped on the Illumina HumanOmniExpressExome chip (Illumina, San Diego, CA) at the RIKEN Center for Integrative Medical Science (Yokohama, Japan). Figure S2 illustrates the CONSORT flow diagram for the TCS. Quality control (QC) was performed as previously described (26). Individuals with pairwise identity by descent (IBD) > 0.125 and excess heterozygosity (F inbreeding coefficient >6 standard deviations from the mean) were excluded, leaving 827 individuals. PCA was performed on the genotype data to quantify genomic ancestry using SMARTPCA (27), revealing 713 genetically European individuals. Out of those, 680 had

complete phenotypic data (CIPN20, cisplatin dose, demographics), and composed the study sample. SNPs with call rate < 0.99 and Hardy-Weinberg equilibrium (HWE) P < 1×10^{-6} were excluded, leaving 930,450 SNPs. These comprised the input set of genotype imputation with SHAPEIT phasing, which we performed on the University of Michigan Imputation Server using the EUR (European) population in 1000G Phase 1v3 ShapeIt2 (no singletons) reference panel (28). 5,068,489 SNPs with minor allele frequency (MAF) > 0.05, imputation R^2 > 0.8 and INFO score 0.6–1.05 were retained for further analysis. No genetic PC was associated with CisIPN in univariate or multivariate analyses investigating the first 10 PCs.

Independent SNP Analysis

We performed independent SNP association tests with CisIPN by proportional odds ordinal logistic regression adjusted for covariates and assuming linear additive SNP effects. These did not include heritable covariates significantly associated with CisIPN, because they can bias effect estimates (29). R version 3.2.0 was used.

GCTA and PrediXcan Analysis

We estimated narrow-sense heritability using a variance-component model with a genetic relationship matrix (GRM) estimated from genotypes using GCTA (30). SNPs with HWE P > 0.05 and MAF > 0.05 and one of a pair of individuals with estimated relatedness >0.025 were included, leaving 4,897,434 SNPs and 623 individuals. We performed gene-based associations using PrediXcan (31). Briefly, PrediXcan uses reference transcriptome data to generate models 'imputing' gene expression in patients from their genotype data by leveraging the effects of SNPs predicting expression. In this study, we used models derived from the application of elastic net ($\alpha = 0.5$). Genome-wide expression was predicted using reference transcriptome panels in four candidate tissues, chosen for their representation of the nervous system and the microenvironment of peripheral nerves and endings: whole blood using Depression Genes and Networks (DGN) (32), and three tissues from GTEx (33): tibial nerve, skin (not exposed to sun), and cerebral cortex. DGN (instead of GTEx) was used as a reference set for whole blood due to the larger sample size (n = 922 vs. 338). Genes with prediction R^2 > 0.01 and prediction p < 0.05 were included. Genes with low-variance predicted expression were excluded ($\sigma^2 < 0.001$). Associations were obtained by ordinal regression as described, with predicted expression as the independent variable and age as a covariate. Genome-wide significance was set at $\alpha = 0.05$ with Bonferroni correction for the number of genes tested within the tissue. Experiment-wide significance was set conservatively with Bonferroni correction for the total number of tests performed in all four tissues.

Replication

To replicate our findings, we used two additional independent datasets, Vanderbilt's electronic health records (EHR) BioVU (34, 35) and the CALGB 90401 trial (36). The BioVu cohort was composed of 18,620 genotyped individuals that were linked to EHR (34) and given a PheWAS code (ICD9-derived codes) for *polyneuropathy due to drugs* (n=20, ICD-9 code 316.1). Association with predicted gene expression was evaluated by one-tailed logistic regression in the direction of the discovery. For CALGB 90401, pre-imputation

genotype quality control was performed with 543,834 SNPs and 623 genetically European individuals as described (36). Genotypes were imputed using IMPUTE2 software and the 1000 Genomes phase 3 EUR reference population. 3,031,145 SNPs with MAF > 0.05, IMPUTE2 INFO score > 0.3 were retained for further analysis. We evaluated the association of predicted gene expression with cumulative docetaxel dose (adjusted for competing-risks) at the time of occurrence of grade 3 or higher neuropathy using one-tailed Cox proportional hazards regression (36). Replication significance was assessed with Fisher's combined p-value method using the one-tailed p-values of the two independent replications at $\alpha = 0.05$.

RESULTS

Cohort Characteristics

The clinical and demographic characteristics of the genetically European subjects (n = 680) that passed all QC and had full phenotypic data are shown in Table 1 (additional diagnosis and treatment characteristic are listed in Table S2). Median age at testicular cancer diagnosis was 31 years (range: 15–50) with a median of 4.8 years (range: 0.4–30) between treatment completion and clinical evaluation. Chemotherapy regimens consisted largely of bleomycin, etoposide, and cisplatin (62.9%) or etoposide and cisplatin (29.9%). At the time of enrollment, one in three patients (33.6%) were either obese (BMI: 30 to 39) or morbidly obese (BMI 40) and only 16.8% participants reported having excellent health (Table S2 and Table 1). Current use of prescription medications for hypertension, hypercholesterolemia, and diabetes was reported by 12.8%, 11.0%, and 3.1% of participants, respectively. Approximately 42% of patients are former (34.4%) or current smokers (7.5%). For those, the median number of years smoking is 5, and the mean is 8.6.

Phenotype Analysis

The EORTC-CIPN20 scale questionnaire (20) included nine sensory, eight motor and three autonomic items (Table S1). More than one third (36–38%) of patients reported experiencing some degree of tingling or numbness in hands and/or feet within the four weeks preceding clinical evaluation, with a smaller proportion (8–14%) reporting shooting pain, difficulty distinguishing hot and cold water, and problems feeling the ground (Figure S3). PCA indicated that items generally clustered by symptom type on the first two Principal Components (PCs), except for hearing loss (Figure S4). In the third and fourth PC, sensory and motor items clustered by limb (i.e., fingers/hands vs. toes/feet). Cumulatively, the four PCs explained 60% of the total phenotypic variance. Autonomic items and the hearing loss item formed a distinct cluster on both plots. We have previously comprehensively evaluated hearing loss in this cohort with quantitative audiometry (37) and assessed genetic variants associated with cisplatin-induced ototoxicity (26). Therefore, we excluded hearing loss item from the sensory subscale. The eight remaining items indicated high levels of internal consistency (Cronbach $\alpha = 0.88$). We created a summary statistic of CisIPN using those items as described. Overall, more than half (56.2%) of patients reported at least some degree of sensory neuropathy, while motor symptoms were less common overall with 45.2% experiencing some form of motor neuropathy and 2.8% with quite a bit/very much (Figure 1) consistent with the observation that cisplatin predominantly causes sensory neuropathy (3).

CisIPN Association with Diagnosis and Treatment Characteristics

Summaries of statistical associations between evaluated variables and CisIPN are presented in Table 2A. CisIPN was associated with age at diagnosis (OR = 1.06, p = 2×10^{-9}). BMI at evaluation did not correlate with CisIPN. Type of chemotherapy regimen (EP vs. BEP [data not shown]) and cumulative cisplatin dose (range: 100–800 mg/m^2) were not significantly associated with CisIPN. Importantly, the number of years since therapy did not correlate negatively with CisIPN (p = 0.43) in age-adjusted analyses, suggesting there is no attenuation of symptoms over time several years following chemotherapy (data not shown). A longitudinal study or one with baseline data remains more appropriate to assess symptom progression.

CisIPN Association with Clinical and Behavioral Characteristics at Evaluation

Age at evaluation significantly correlated with CisIPN (OR = 1.04, p = 2×10^{-8}) but displayed a slightly smaller effect size than at diagnosis (Table 2B). Age at diagnosis and evaluation were highly correlated ($R^2 = 0.8$), and therefore, subsequent analyses were adjusted for age at diagnosis only. Smoking status ('ever smoker') (OR = 1.54; p= 0.004) as well as the self-reported number of years smoking (OR = 1.04 per year, p = 0.0002 [data not shown]) and excess drinking (OR = 1.83; p = 0.007) were significantly associated with CisIPN in univariate, age-adjusted analyses. CisIPN was also negatively correlated with physical activity level (OR = 0.72; p = 0.02; data not shown). Hypertension displayed a correlation (OR = 1.61, p = 0.03) with CisIPN, but not hypercholesterolemia (OR = 0.92, p = 0.72) or diabetes (OR = 1.27, p = 0.59), although the sample size of the latter was small (n=21). In the multivariate model, hypertension was marginally associated with CisIPN while age, smoking status, and excessive drinking displayed consistent effect sizes and statistical significance (Table 2C). BMI at evaluation was marginally associated with CisIPN (OR = 1.03, p = 0.051). Weight gain as measured by the absolute difference in BMI at evaluation and BMI at treatment initiation showed a stronger and more significant association with CisIPN (OR per kg/m^2= 1.05; p = 0.004, adjusted for years since treatment, Figure 2A).

CisIPN Association with Self-Reported Health

In a univariate analysis, we found a strong negative correlation of CisIPN with self-reported health (OR = 0.56; p = 2.6×10^{-9}) (Figure 2B). A multivariate model with self-reported health as the dependent variable revealed a negative correlation with CisIPN (OR = 0.59, p < 0.0001) and with weight gain since treatment (OR = 0.91, p < 0.0001), and a positive correlation with physical activity (25) (OR = 1.92, p < 0.0001). Considering additional variables, self-reported health negatively associated with smoking (OR = 0.64, p= 0.003) but not hypertension (OR = 0.69, p = 0.096) or excess drinking (OR = 0.70, p = 0.12). CisIPN (OR = 0.64, p < 0.0001), weight gain (OR per kg/m^2= 0.91, p < 0.0001), and exercise (OR = 1.97, p < 0.0001) remained the most significantly associated variables in a model including hypertension and smoking. We compared these findings in CisIPN to ototoxicity on the subset of TCS (n = 511) with quantitative audiometry included in a prior report (37), using a rank normalized geometric mean of audiogram thresholds in linear regression, adjusted for age. Although cisplatin-associated hearing loss and CisIPN were correlated (β =

0.14, p = 0.008), there was no relationship between ototoxicity and weight gain, physical activity, or self-reported health (data not shown, p > 0.05).

GWAS of CisIPN

The results of the GWAS using the 5,068,489 SNPs with age as a covariate are shown in Figure S5, and the top 100 SNPs in Table S3. No SNP met genome-wide significance (p < 5 × 10^{-8}). None of the four SNPs in *GSTP1* (18, 38), *ABCG2* (39), *XPC* (39), and *ERCC1* (15) previously identified in candidate gene studies of peripheral neuropathy following platinum-based regimens were replicated (Table S4). Out of the four SNPs meeting genome-wide significance in previous GWAS of CIPN due to other neurotoxic chemotherapies (Table S5), one SNP (rs7349683 in *EPHA5*) met replication significance (p = 0.05) with a concordant direction of effect. A SNP in ATP8A2 (rs1326116) that was of borderline significance (p=1.8 × 10^{-6}) in a GWAS of docetaxel-induced neuropathy (36) showed the same direction of effect and met replication significance (p= 0.01).

GCTA and PrediXcan Analysis

Using GCTA (30), we found CisIPN to be highly heritable, with common variants explaining up to 74% of phenotypic variability (h^2 = 0.74, SE = 0.48; p = 0.03). This finding suggests a polygenic architecture of CisIPN. We used PrediXcan, a gene based test (31), to associate genetically regulated components of gene expression with CisIPN (Figure 3). This method increases power to explain the heritability by aggregating the effects of SNPs associated with gene expression. The most significant (p <0.001) results are listed in Table S6. One gene met experiment-wide significance (*MIDN* in skin, p = 1.4 × 10^{-6}) and two genes met genome-wide significance (*RPRD1B* in whole blood, p = 3.4 × 10^{-6} and *THEM5* in tibial nerve, p = 4.9 × 10^{-6}). *RPRD1B* revealed a protective effect, i.e. lower *RPRD1B* gene expression is associated with CisIPN (Figure 4A). *MIDN* had the same direction of effect, while *THEM5* displayed the opposite. Although *RPRD1B* was discovered using DGN, it was the second most significant hit in both GTEx skin and tibial nerve tissues.

In efforts to replicate, we interrogated a BioVu cohort of 18,620 genotyped individuals that were linked to EHR (34, 35) with the phenotype "polyneuropathy due to drugs" and found lower PrediXcan-predicted *RPRD1B* expression was associated with this phenotype (Figure 4B, one-tailed p = 0.021, 20 cases, 18600 controls). We further investigated the association in the Cancer and Leukemia Group B (CALGB) 90401 cohort, an independent dataset assessing docetaxel-induced peripheral neuropathy and found it nominally significant (Figure 4C, HR = 0.4, one-tailed p = 0.055). We assessed replication significance by computing the combined p-value using Fisher's method and found replication to be significant (p = 0.0089).

DISCUSSION

Although cisplatin is one of the most commonly used cytotoxic drugs worldwide, to our knowledge, this study represents the first and largest investigation comprehensively evaluating clinical and genome-wide associations with long-term CisIPN in TCS, an ideal population for long-term pharmacogenetic studies of cisplatin toxicities. The validated

EORTC-CIPN20 was applied in this large series to capture a broad spectrum of neurological damage from the patient's perspective that might not be apparent on formal neurologic testing (40), and confounded by interpretation in physician-graded scales (41). We found sensory CisIPN symptoms to be common, and significantly correlated with weight gain since treatment, and inversely correlated with self-reported health and physical activity. In univariate models, we identified age, smoking status, excess drinking, and hypertension as significantly associated with CisIPN. As these variables are non-independent, we assessed their associations in a multivariate model and found some of the effect of hypertension to be explained by the other variables. The cross-sectional design prevents conclusions as to whether these associated variables are risk factors or consequences of CisIPN which would require a prospective design. We found significant heritability, indicating that genetic variants could explain a large proportion of variability in the phenotype. Aggregating SNP effects using PrediXcan (31), a gene-level test imputing genetically regulated expression from genotypes, revealed lower expression of *MIDN* in skin and *RPRD1B* in whole blood, as well as higher expression of *THEM5* in tibial nerve as genome-wide significant. Using two independent datasets, we replicated the finding that lower *RPRD1B* predicted expression is associated with drug-induced neuropathy using predictors from whole blood, investigating a docetaxel-induced neuropathy phenotype and a broader drug-induced polyneuropathy phenotype in EHR.

PrediXcan

PrediXcan is a recently developed gene-based method that tests the genetically determined component of gene expression in a given tissue for association with a phenotype (31). The use of genetically determined gene expression to identify trait-associated genes is supported by evidence that SNPs associated with chemotherapeutic drug susceptibility (42) and complex traits (43) are more likely to be sites that associate with gene expression. Further, an observed association with the genetic component proposes a causal direction of effect since the phenotype does not alter the germline genetic profile. We theorized that tibial nerve, skin, and whole blood best represent neuronal tissue and the microenvironment surrounding nerves and their endings, a potentially important source of variability in CIPN. Since genetic regulation of gene expression can be non-tissue specific, the Bonferroni correction (correcting for the total number of tests across the tissues) is conservative. In three of the four tissues tested, lower expression of *RPRD1B* is associated with CisIPN at p $< 10^{-4}$, illustrating substantial tissue-shared genetic regulation. Vanderbilt's BioVU identified lower predicted gene expression of *RPRD1B* in the discovery tissue (DGN whole blood) as associated with drug-induced polyneuropathy. However, the analysis could not distinguish drug classes. Further suggestive association of neuropathy with lower expression of *RPRD1B* in the CALGB 90401 may indicate that the mechanism for the association is not drug-specific, as that trial investigated neuropathy induced by docetaxel, another neurotoxic chemotherapeutic agent with a distinct mechanism of cytotoxicity. Therefore, *RPRD1B* might have broad implications in CIPN.

RPRD1B

Twenty SNPs in four distinct linkage disequilibrium blocks ($R^2 < 0.5$) 36–37.5 kilobases into chromosome 20 predict *RPRD1B* expression. *RPRD1B* codes for Kub5-Hera, a protein

regulating the binding of RNA polymerase II to *CCND1* gene (cyclin D1), and regulating the transcription of several genes involved in the cell cycle (44). Emerging data indicate that *RPRD1B* plays an important role in several DNA repair mechanisms, including double-strand breaks (DSB) repair through the association with core non-homologous end joining (NHEJ) proteins (45) and mismatch repair (46). Defects in *RPRD1B* expression or knockdown cause a deficiency in DNA repair mechanisms known to be critical in resolving cisplatin-induced lesions (47). Consistent with our data indicating low levels of *RPRD1B* being associated with CisIPN, knockdown of this gene in a breast cancer cell line, MDA-123 results in increased sensitivity to cisplatin (45). However, the suggestive association of *RPRD1B* with docetaxel-induced neuropathy, indicates that other mechanisms may be important. *RPRD1B* functions as a co-activator of the β-catenin-TCF complex to enhance the transcriptional activity of Wnt signaling (48). Wnt signaling is critical for initial neural cell-fate determination, patterning and synapse formation of sensory neurons of the dorsal root ganglia. This signaling pathway is also active in adult sensory neurons and modulates sensitivity to nociceptive stimuli (49, 50). Mechanistic insights into the function of RPRD1B are warranted to assess its role in the pathophysiology of neurotoxicity, possibly revealing novel targets.

Demographic and clinical factors and health behaviors

Our study identified age, smoking, excess alcohol use, and hypertension as associated with CisIPN. The relationship with age is consistent with several previous reports of taxane-induced neuropathy, including ECOG 5103 (4, 8, 36). In contrast, a number of studies of oxaliplatin-treated patients did not find an association with age (6, 7).

Tobacco and alcohol use—Few studies have addressed the role of tobacco use in CIPN. The authors of one study that found a correlation between smoking and CIPN postulated that long-term heavy smoking reduces peripheral blood flow, likely exacerbating paclitaxel-induced neuropathy (5). Conversely, a study of 730 TCS given platinum-based chemotherapy (9) and another of 62 colon cancer patients receiving oxaliplatin reported no association (7). Among 169 patients given oxaliplatin-based chemotherapy, alcohol consumption was associated with neuropathy (6). In contrast, two studies in breast cancer patients found no such relation of CIPN (10, 11). Alcohol-related peripheral neuropathy is a complication of alcoholism affecting up to half of these individuals (51). Excess alcohol use also plays a role in the development and progression of diabetic neuropathy (52).

Hypertension—In our study, the association between hypertension and CIPN was of borderline significance in multivariate analyses. Hypertension was not significantly associated with CIPN in the studies by Hershman et al. (4) and Glendenning et al. (9). One could postulate that microvascular complications associated with hypertension may contribute to CIPN, but our findings remain to be confirmed in other studies.

Lower physical activity and weight gain—We found CisIPN was associated with lower physical activity levels and greater weight gain since chemotherapy completion. While a longitudinal study design would be best for establishing causal inferences, it is possible that CIPN symptoms could deter from physical activity and thereby promote weight gain.

Known downstream effects of weight gain include obesity, diabetes and other medical complications. Another long-term study in TCS has shown other co-morbidities are associated with CIPN, including neuroticism (53), further emphasizing the clinical impact of CIPN symptoms and their effects on patient health.

Strengths and limitations

A major strength of our study consists of its comprehensive assessment of a variety of clinical and genetic factors associated with CisIPN. Other strengths include the homogeneity of cisplatin-based chemotherapy without the administration of other neurotoxic drugs, detailed data on cisplatin dose, and the high patient participation rate (93%). Patient reported outcomes were carefully considered, and for the CisIPN phenotype, the validated EORTC QLQ-CIPN20 questionnaire (20) was applied. Although a number of studies of CIPN (reviewed above) considered the influence of one or a few covariates on CIPN, the present investigation considered all variables taken together. In addition, to our knowledge, no other study has considered the impact of CIPN on self-reported health or reported associations with weight gain and physical activity. PrediXcan (31), a state-of-the-art gene based method implicated *RPRD1B*, a gene known to play an important role in DNA repair mechanisms involved in cisplatin-induced damage, and shown to increase cisplatin sensitivity upon knockdown (45). Predictions are most helpful for variables known prior to treatment, yet as in previous studies (15, 18, 38, 39), baseline data before treatment were not collected. Any cross-sectional study design has potential inherent limitations, and does not allow us to infer causation of other evaluated risk factors for CisIPN, but rather to report important associations.

Another limitation in this study is the underpowered statistical analyses, as only 85 out of 680 reported severe CisIPN, although a larger proportion reported moderate symptoms (297 of 680). A larger sample size would allow better estimation of the effects of clinical factors and SNPs, and would enable making meaningful inferences about phenotype heritability which is limited by large standard errors here. Last, in replicating our study in a large set of EHR, we found lower predicted gene expression of *RPRD1B* to be associated with *drug-induced polyneuropathy*; however this phenotype was not able to distinguish specific drug classes.

Conclusions

In view of the significant associations we found between smoking, excess alcohol use, decreased physical activity, and weight gain with CisIPN, health care providers should promote a healthy lifestyle among patients with CisIPN. The borderline significant association we observed for hypertension and CisIPN remains to be confirmed in other studies, but nonetheless, in view of the highly significant relationship we previously reported between hypertension and hearing loss in TCS (37), health care providers should carefully monitor blood pressure.

There are currently no agents available to prevent or treat CIPN (12), which in our population and as reported by others (9) persists long-term for cisplatin. This observation underscores the importance of identifying at-risk patients prior to the administration of

chemotherapy, which is currently not possible. Significant heritability indicates that a large proportion of variability in toxicity could be explained by genetic variants, but larger samples are needed to reduce the standard error before variants can be used in clinical prediction. Future research efforts should continue to elucidate the genetic underpinnings of CIPN, with our investigation now showing the importance of using functional genomic information such as expression data in genome-wide analyses to improve power and provide mechanistic explanations. Such approaches are more robust than reporting single polymorphisms with small-to-moderate effect sizes, which require larger datasets and are less clinically impactful. In addition, future research efforts should continue to provide the underpinnings for the eventual development of risk prediction models for CIPN that take into account not only genetic influences, but also clinical, demographic, and other important covariates. Our study provides one such example of a comprehensive approach. Future efforts will focus on independent replication in a similarly characterized TCS cohort, and evaluation of the predictive power of the variables associated in this study to potentially translate models of CisIPN prediction into the clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

CIPN	chemotherapy-induced peripheral neuropathy
CisIPN	cisplatin-induced peripheral neuropathy
TCS	testicular cancer survivors

EORTC QLQ-CIPN20	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Chemotherapy Induced Peripheral Neuropathy20
QoL	quality of life
GCT	germ cell tumor
BMI	body mass index
PCA	principal component analysis
MET	Metabolic Equivalent of Task
EUR	European
IBD	identity by descent
GTEx	Gene-Tissue Expression project
DGN	Depression Genes and Networks
OR	odds ratio

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Translational Relevance

Cisplatin is one of the most commonly prescribed chemotherapeutics worldwide, but is associated with multiple toxicities that adversely impact survivors' quality of life. One such toxicity, CisIPN often persists and can be debilitating. We identified strong effects of long-term CisIPN on self-reported health and showed associations of CisIPN with age, hypertension and modifiable risk factors such as smoking, excess drinking and weight gain since treatment. We found CisIPN to be highly heritable, indicating that genetic variants could explain phenotypic variability. We implicated lower (genetically determined) expression of *RPRD1B* and *MIDN* and higher expression of *THEM5* as associated with CisIPN. *RPRD1B* was replicated in an independent cohort of patients who developed drug-induced polyneuropathy and was of borderline significance in a clinical trial of docetaxel-induced neuropathy. *RPRD1B* functions in DNA repair, transcription, and cell cycle control and may be a target for drug development. Our data indicate the importance of both clinical characteristics and genetic variation in the development of long-term CisIPN.
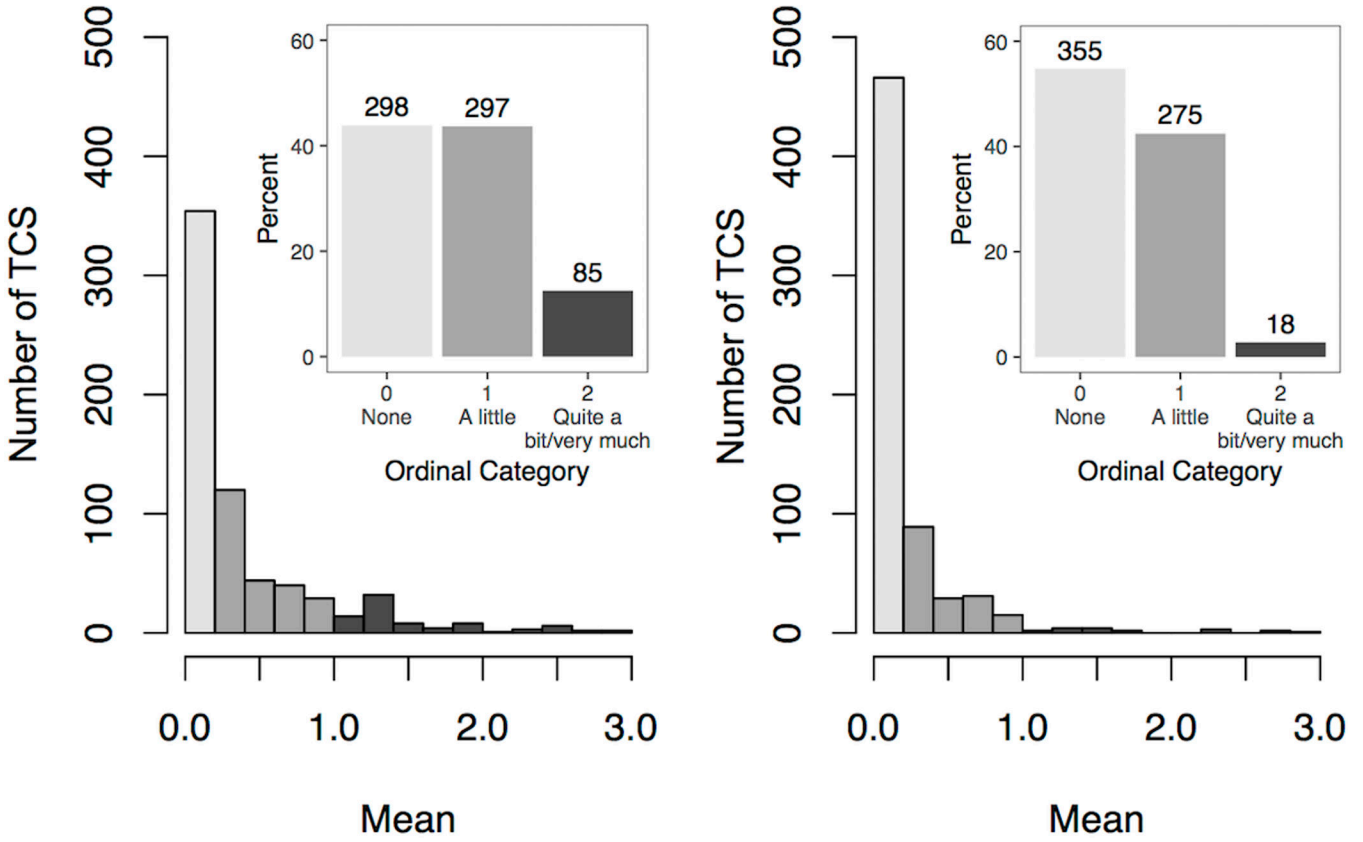
Figure 1. Distributions of summary statistics for A) the sensory subscale (eight items), and B) the motor items (eight items)

Outset: Following the conversion of the Likert "none"-"very much" scale to a 0–3 numeric scale, each individual was attributed a summary statistic for the sensory subscale (Cronbach $\alpha = 0.88$) and the motor subscale ($\alpha = 0.78$) by taking the mean of the responses in the subscale: none (0; mean = 0), a little (1; 0 < mean 1), quite a bit (2; 1 < mean 2), very much (3; 2 < mean 3). **Inset:** Percent of patients in each group (top of column lists actual number of patients) with groups 2 and 3 combined due to low frequency.
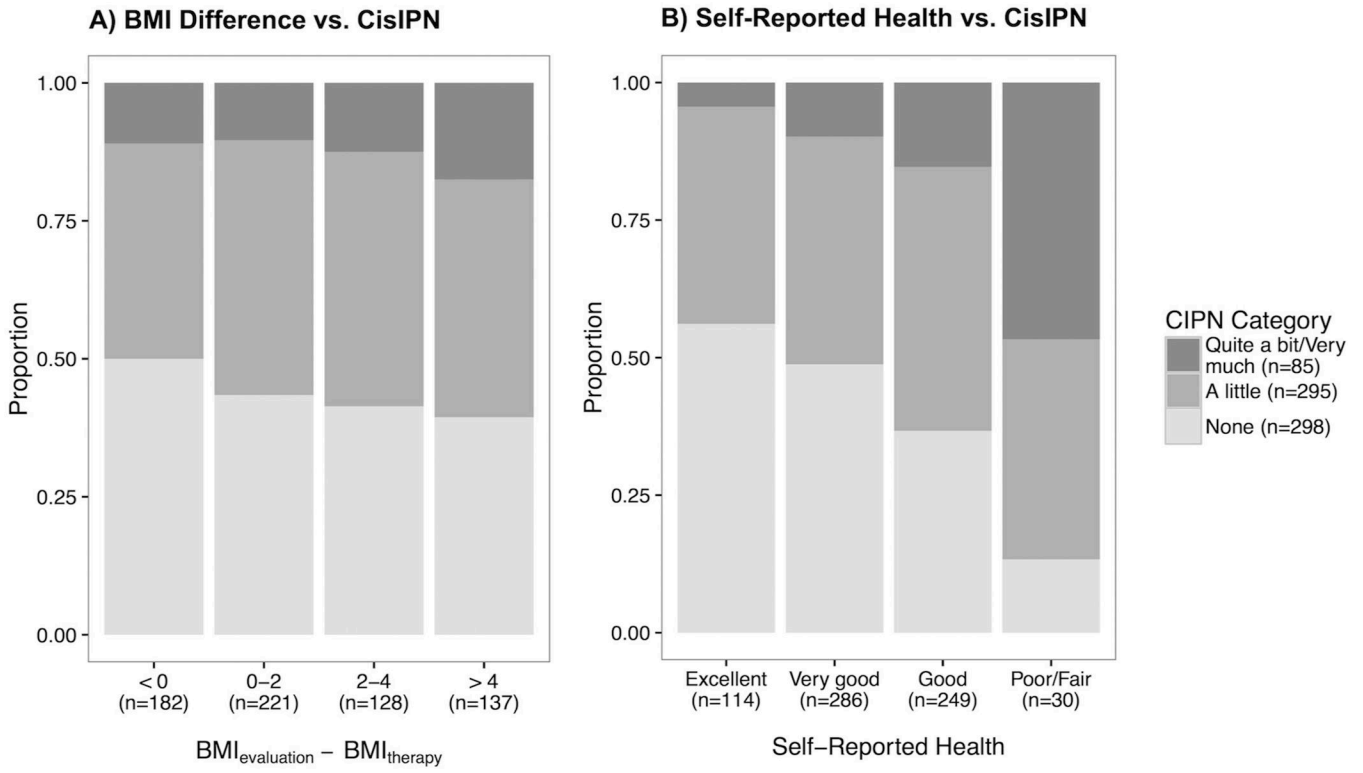
Figure 2. Relationship between weight gain and self-reported health, and severity of CisIPN
Barplots of CisIPN vs **A.** Weight gain (measured as the BMI difference between evaluation and therapy) and **B.** Self-reported health. BMI difference positively correlated with CisIPN (OR = 1.05) after adjusting for age (p = 0.009) or number of years since treatment (p = 0.004). Self-reported health (poor-excellent) strongly negatively correlated with CisIPN (OR = 0.56, p = 2.6×10^{-9}), but not with age.
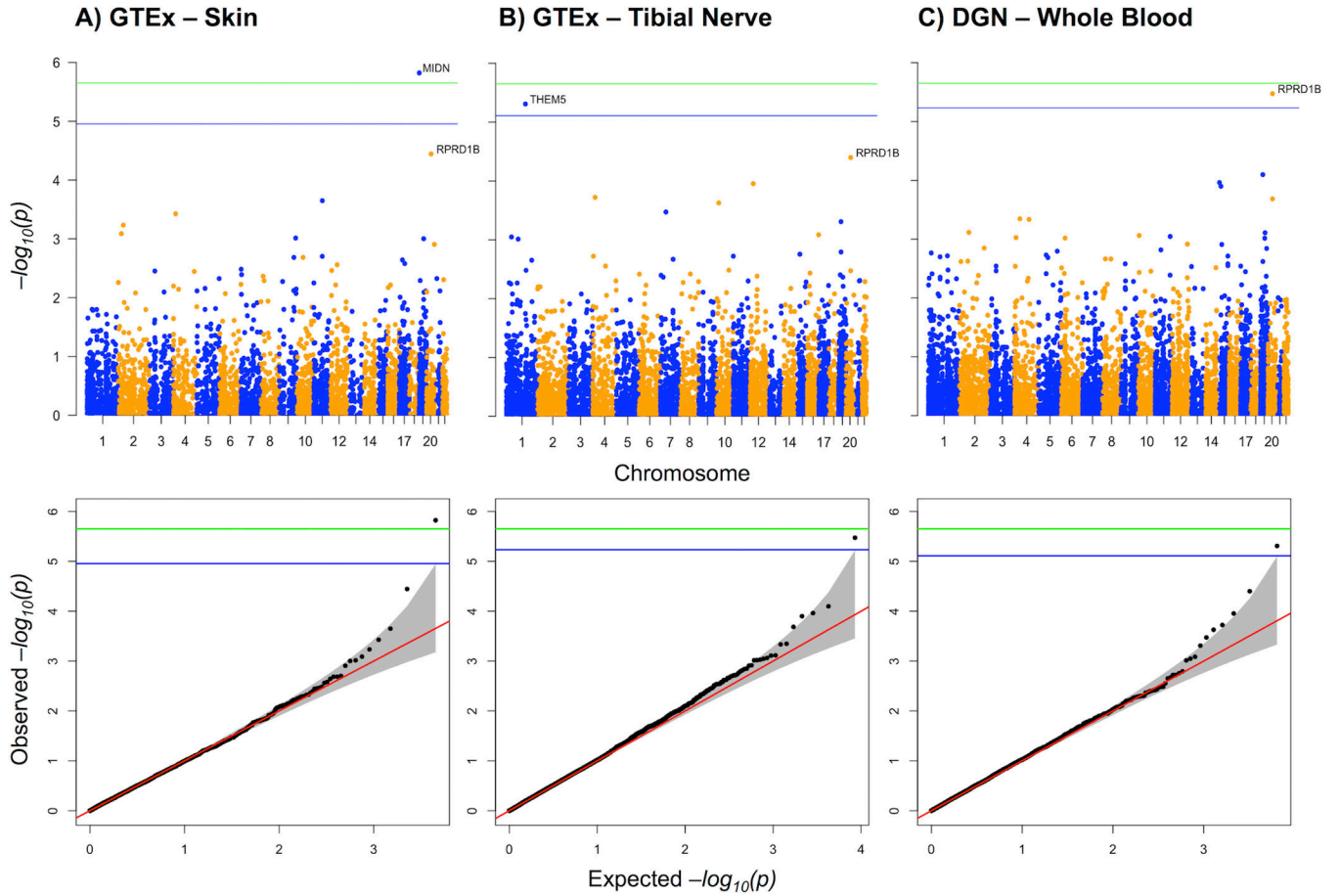
Figure 3. Genome-wide PrediXcan analysis of CisIPN in TCS

Manhattan and Q-Q plots of associations with PrediXcan expressions in 3 out of 4 candidate tissues tested, including **A.** GTEx skin – not exposed to sun, **B.** GTEx tibial nerve, **C.** DGN whole blood. Genes that passed the following criteria were included: prediction $R^2 > 0.01$, prediction $p < 0.05$, predicted expression variance > 0.001. Blue lines indicate significance threshold within the tissue, $-\log_{10}(0.05/n_{tissue})$ where n_{tissue} is the number of genes tested in the tissue. The green line indicates experiment-wide significance threshold, $-\log_{10}(0.05/n_{total})$ where n_{total} is the total number of tests performed.
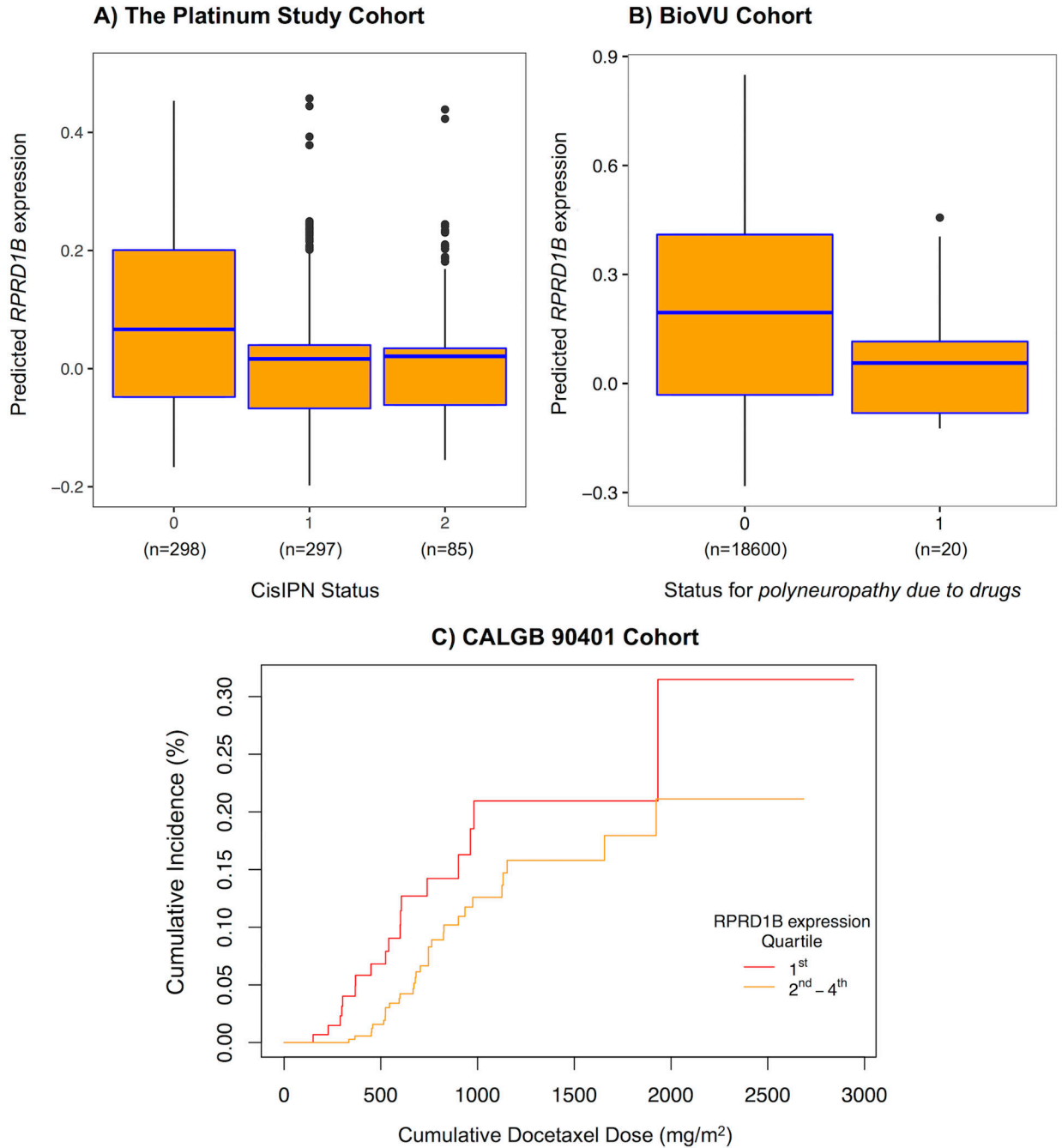
Figure 4. Association of lower *RPRD1B* expression and CisIPN

Box and cumulative incidence plots of PrediXcan-predicted *RPRD1B* expression by
neuropathy status reveal that lower expression correlates with neuropathy in **A.** The
Platinum Study's TCS cohort (p = 3.6 × 10⁻⁶), and **B.** Vanderbilt's BioVU cohort (one tailed
p = 0.021) and **C.** the CALGB 90401 docetaxel trial (one tailed p = 0.055). In plots A and B:
The centers of the boxplots indicate means, the hinges indicate interquartile regions (IQR),
the whiskers indicate points within 1.5 × IQR. Data beyond the end of the whiskers are
outliers plotted as points. In plot B: Logistic regression was performed in BioVU to assess

the association between PrediXcan expression and the code for *polyneuropathy due to drugs* – 1 for cases (n = 20), 0 for controls (n = 18,600). In plot C: Cox proportional hazards regression was performed to assess the association between PrediXcan expression and a dose-to-grade 3 or higher neuropathy event in the CALGB. An arbitrary cutoff is used to illustrate the association between the continuous gene expression variable and the dose-to-event phenotype: Individuals were ranked according to gene expression as determined by PrediXcan and the 1st quartile refers to the 25% with the lowest genetically determined *RPRD1B* expression and 2nd to 4th refers to the remaining individuals. Replication was assessed by Fisher's combined p-value of both replications (BioVu and CALGB) and met significance (p = 0.0089).

Table 1

Demographic features and clinical characteristics of 680 genetically European male germ cell tumor (GCT) survivors at the time of clinical evaluation

Characteristic	Number (%)
Total patients	680
Age at clinical evaluation (years)	
Median (range)	38 (18–68)
<20 y	5 (0.7%)
20–29 y	124 (18.2%)
30–39 y	251 (36.9%)
40–49 y	186 (27.4%)
50 y	114 (16.8%)
Self-reported race	
White	660 (97.1%)
Non-white	20 (2.9%)
Chemotherapy regimen	
Cisplatin, bleomycin, etoposide: total[a]	428 (62.9%)
2 cycles	13 (1.9%)
3 cycles	282 (41.5%)
4 cycles	124 (18.2%)
5 cycles	9 (1.3%)
Cisplatin, etoposide: total[b]	203 (29.9%)
3 cycles	2 (0.3%)
4 cycles	196 (28.8%)
5 cycles	5 (0.7%)
Other cisplatin-based regimen: total[c]	49 (7.2%)
3 cycles	6 (0.9%)
4 cycles	39 (5.7%)
5 cycles	4 (0.6%)
Cumulative dose of cisplatin (mg/m^2), all patients[d]	
<300	36 (5.3%)
300	258 (37.9%)
301–399	28 (4.1%)
400	328 (48.2%)
>400	30 (4.4%)
Time from completion of chemotherapy to clinical evaluation, (years)	
Median (range)	4.8 (0.4–29.9)
<2	146 (21.5%)
2–5	250 (36.8%)

Characteristic	Number (%)
6–9	127 (18.7%)
10	156 (22.9%)
Not available	1 (0.1%)
Hypertension and on prescription medication[e]	
Yes	87 (12.8%)
No[f]	593 (87.2%)
Diabetes and on prescription medication[g]	
Yes	21 (3.1%)
No[h]	659 (96.9%)
Hypercholesterolemia and on prescription medication[i]	
Yes	75 (11.0%)
No[j]	605 (89.0%)
Smoking status	
Current smoker	51 (7.5%)
Former smoker	234 (34.4%)
Never smoker	395 (58.1%)
Average number of alcoholic drinks in past year	
<2 drinks per day	593 (87.2 %)
2 drinks per day	84 (12.4 %)
Not indicated	3 (0.4%)
Self-rating of health[k]	
Excellent	114 (16.8%)
Very good	286 (42.1%)
Good	249 (36.6%)
Poor/Fair[l]	30 (4.4%)

[a] Median cumulative cisplatin dose for BEP-treated patients was 300 mg/m^2 (range: 200–800): 300 mg/m^2 (range: 272–400) among those receiving 3 cycles, and 400mg/m^2 (range: 344–653) among those receiving 4 cycles. 317 received the standard doses for each cycle (i.e., bleomycin 30 units IV weekly, etoposide 100 mg/m^2 IV daily × 5 days, cisplatin 20 mg/m^2 IV daily × 5 days) and 111 received a modified dose for at least one cycle.

[b] Median cumulative cisplatin dose for EP-treated patients was 400 mg/m^2 among patients receiving 4 cycles (range: 400–600), and among all patients (range: 200 – 600). Of patients receiving EP, 129 received standard dose (etoposide 100 mg/m^2 IV daily × 5 days, cisplatin 20 mg/m^2 IV daily × 5 days) and 74 received a modified dose for at least one cycle.

[c] Of 49 patients, 24 received cisplatin, etoposide, ifosfamide regimen (17 standard, 7 modified), 14 received combination chemotherapy consisting of cisplatin and ifosfamide; 3 received cisplatin, bleomycin, etoposide, and ifosfamide, 1 patient received VelP, 1 patient received PVB, and 1 patient received unknown cisplatin-based regimen. For the remaining 5 patients, other combinations of cisplatin-based chemotherapy were applied.

[d] Median cumulative dose of cisplatin among all patients was 400 mg/m^2 (range: 200–800).

[e] Includes patients who answered "Yes" to 1) have you ever been diagnosed with high blood pressure and "Yes, current" to 2) have you ever taken prescription medications for high blood pressure (including current use).

[f] Includes 5 patients for whom status of either hypertension diagnosis or current prescription medication use was not reported.

g Includes patients who answered "yes" to either of the following questions: 1) diabetes requiring insulin or 2) diabetes requiring tablets or pills.

h Includes 13 patients for whom status of diabetes and current prescription medication use was not reported.

i Includes patients who answered "yes, current" to the following question: have you ever taken prescription medications for high cholesterol.

j Includes 3 patients for whom status of hypercholesterolemia and on prescription medication use was not reported.

k Self-rating of health was not indicated by one patient.

l Out of 30 patients, 25 assigned fair and 5 assigned poor to their self-rating of health.

Table 2

Association of treatment and other variables with cisplatin-induced peripheral neuropathy. Bolded variables are significantly associated at $\alpha = 0.05$.

A. Treatment Variables			
Model	**Variable**	**OR (95% CI)**	**P-value**
CisIPN ~ Variable	**Age at diagnosis**	**1.06 (1.05–1.07)**	**2×10^{-9}**
CisIPN ~ Variable (+Age$_{diagnosis}$)	BMI at treatment	1.00 (0.97–1.03)	0.96
	Cisplatin dose[a] (mg/m^2)	1.00 (0.99–1.00)	0.39
B. Variables at Clinical Evaluation			
CisIPN ~ Variable	**Age at evaluation**	**1.04 (1.03–1.06)**	**2×10^{-8}**
CisIPN ~ Variable (+Age$_{diagnosis}$)	**Smoking[b]**	**1.54 (1.15–2.07)**	**0.004**
	Excess drinking[c]	**1.83 (1.18–2.84)**	**0.007**
	Hypertension[d]	**1.61 (1.04–2.50)**	**0.03**
	BMI at evaluation	1.03 (1.00–1.05)	0.051
	Diabetes[e]	1.27 (0.54–2.95)	0.59
	Hypercholesterolemia[f]	0.92 (0.57–1.48)	0.72
C. Multivariate model			
CisIPN ~ Significant Variables	**Age$_{diagnosis}$**	**1.04 (1.02–1.06)**	**3×10^{-6}**
	Excess drinking	**1.82 (1.16–2.86)**	**0.009**
	Smoking	**1.56 (1.15–2.10)**	**0.004**
	Hypertension	1.54 (0.97–2.42)	0.065

[a] Dose group was an ordinal variable created from cumulative cisplatin doses (in mg/m^2) classified as follows: <300, 300–400 (excluding 400), 400–500 (excluding 500), and 500. OR is based on ordinal group.

[b] Includes patients who answered yes to the question "have you ever smoked cigarettes?"

[c] Includes patients who answered answered 2 drinks/day to the question "during the past year, how many drinks of alcoholic beverage have you consumed?"

[d] Includes patients who answered yes to both of the following: 1) "have you ever been diagnosed with high blood pressure?" and 2) "are you currently taking prescription medication for high blood pressure?"

[e] Includes those patients who answered "yes" to either of the following questions: 1) "diabetes requiring insulin?" or 2) "diabetes requiring tablets or pills?".

[f] Includes those patients who are currently taking prescription medications for high cholesterol.