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Pharmacoethnicity in Paclitaxel-Induced Sensory Peripheral Neuropathy

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Abstract

Purpose—Paclitaxel is used worldwide in the treatment of breast, lung, ovarian and other cancers. Sensory peripheral neuropathy is an associated adverse effect that cannot be predicted, prevented or mitigated. To better understand the contribution of germline genetic variation to paclitaxel-induced peripheral neuropathy, we undertook an integrative approach that combines genome-wide association study (GWAS) data generated from HapMap lymphoblastoid cell lines (LCLs) and Asian patients.

Methods—GWAS was performed with paclitaxel-induced cytotoxicity generated in 363 LCLs and with paclitaxel-induced neuropathy from 145 Asian patients. A gene-based approach was used to identify overlapping genes and compare to a European clinical cohort of paclitaxel-induced neuropathy. Neurons derived from human induced pluripotent stem cells were used for functional validation of candidate genes.

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Results—SNPs near *AIPL1* were significantly associated with paclitaxel-induced cytotoxicity in Asian LCLs ($P < 10^{-6}$). Decreased expression of *AIPL1* resulted in decreased sensitivity of neurons to paclitaxel by inducing neurite morphological changes as measured by increased relative total outgrowth, number of processes and mean process length. Using a gene-based analysis, there were 32 genes that overlapped between Asian LCL cytotoxicity and Asian patient neuropathy ($P < 0.05$) including BCR. Upon BCR knockdown, there was an increase in neuronal sensitivity to paclitaxel as measured by neurite morphological characteristics.

Conclusion—We identified genetic variants associated with Asian paclitaxel-induced cytotoxicity and functionally validated the AIPL1 and BCR in a neuronal cell model. Furthermore, the integrative pharmacogenomics approach of LCL/patient GWAS may help prioritize target genes associated with chemotherapeutic-induced peripheral neuropathy.

Keywords

paclitaxel; neuropathy; Asians; GWAS; iPSCs

Introduction

Paclitaxel, a microtubule-stabilizing agent that binds tubulin and disrupts cell division, represents standard of care for common cancers, including breast, lung and ovarian cancer (1). Unfortunately, about 20-30% of patients treated with paclitaxel experience clinically relevant sensory peripheral neuropathy (2, 3). Furthermore, there is substantial inter-patient variability in time to symptom onset, time to peak symptoms, severity of peak symptoms and reversibility (4, 5). Patients experiencing moderate to severe chemotherapy induced peripheral neuropathy (CIPN) report a reduced quality of life, chronic discomfort and disruption of physical abilities for general life activities that can be temporary or permanent (6). CIPN causes a significant symptom burden because many patients that undergo adjuvant treatment are long-term survivors. Moreover, CIPN can lead to dose reduction of the chemotherapeutic agent or possible cessation of treatment (7). This may have an adverse impact on cancer treatment and disease outcomes, particularly in the adjuvant setting.

There are few effective methods in clinical practice for managing peripheral neuropathy. ASCO recommendations, recently published (7) and based on a systematic evaluation of 48 randomized controlled trials, concluded that there are no agents recommended for the prevention of CIPN and supported a moderate recommendation of duloxetine for pain associated with CIPN (8). Physical therapy, acupuncture, massage and medications such as steroids, anti-epileptics, anti-depressants and opioids for pain management are often attempted. Unfortunately, the extent to which these interventions improve symptoms is limited and some of these interventions carry side effects of their own (7). An improved understanding of the genetic variants and genes contributing to paclitaxel induced neuropathy will assist in the development of future neuroprotective agents and in the design of novel therapies with improved toxicity profiles. Furthermore, finding predictive genetic biomarkers could enable identification of patients who should be treated with neuroprotective agents (when they are available) and/or be treated with reduced paclitaxel doses or switched to non-neuropathic treatments.

In attempts to identify genes relevant to CIPN, several genome-wide association studies (GWAS) have been conducted with the identification of single-nucleotide polymorphisms (SNP) in *FGD4* (9), *EPHA5* and *XKR4* (10) associated with the risk of paclitaxel-induced sensory peripheral neuropathy. Because it is challenging to replicate large, well-controlled clinical trials to evaluate pharmacogenomics markers of toxicity (11, 12), we developed an integrative approach combining clinical studies and cell-based models enabling variant identification and followed with functional validation of the findings to help elucidate underlying mechanisms and pathways (13). This method has found utility for paclitaxel-induced peripheral neuropathy in individuals of European descent and capecitabine-induced hand-foot syndrome (14, 15).

Although paclitaxel is used globally, large clinical GWAS studies of paclitaxel-induced sensory peripheral neuropathy have been performed primarily in European patients (9, 10, 14, 16). However, pharmacoethnicity have been recognized as an important factor in chemotherapeutic response and toxicity (17). In this report, we compare genetic variants identified in a GWAS of sensitivity of LCLs derived from individuals of Asian, European and African ancestry to variants identified in an Asian patient GWAS of paclitaxel neuropathy. In addition to analyzing SNPs, we identified significant genes through expression quantitative trait loci (eQTL) data (18) and functionally validate a target gene using human iPSC-derived neurons.

Materials and Methods

Cytotoxicity assays

HapMap LCLs derived from Japanese individuals residing in Tokyo, Japan (HAPMAPPT02/05, JPT, n = 57) and a set derived from Han Chinese residing in Beijing, China (HAPMAPPT02/05, CHB, n = 59), purchased from the Coriell Cell Repositories (Camden, NJ, USA), were treated with increasing concentrations of paclitaxel (Sigma-Aldrich, St. Louis, MO, USA). Cytotoxicity was determined using an AlamarBlue (Invitrogen, nologies Inc.; Carlsbad, CA, USA) cellular growth inhibition assay as described (19). Mean area under the paclitaxel concentration-cell survival percentage curve (AUC) was determined by at least 6 replicates from 2 independent experiments and used as the phenotype in the GWAS. AUC values for each cell line were $\log_2$-transformed before statistical analysis to form an approximately normal distribution. Paclitaxel cytotoxicity data of CEU (n = 77), YRI (n = 87) and ASW (n = 83) are previously published (19). For each population, a linear regression was calculated to determine the correlation between the AUC for each replicate; the correlation for CEU, YRI, ASW, CHB and JPT was 0.83, 0.86, 0.81, 0.83 and 0.82, respectively.

Authentication of Cell Lines

The PAAR Cell Core routinely checks the identity of LCLs by genotyping 47 informative SNPs included in the Sequenom iPLEX Sample ID Plus Panel (San Diego, CA, USA). Using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) DNA was extracted from each sample. Genotyping was performed following the iPLEX Pro application guide and the iPLEX Pro reaction products were dispensed onto a 384-sample SpectroCHIP and

run on a Sequenom MassARRAY system at The University of Chicago DNA Sequencing and Genotyping Facility. Genotypes passed quality control (formed clear genotype clusters) for 46/47 SNPs and all 46 genotypes matched the known HapMap (http://hapmap.ncbi.nlm.nih.gov/) genotypes for all samples. Twenty-four of 116 LCLs were randomly chosen from this study and all passed quality control.

LCL genome-wide association studies

A GWAS of paclitaxel-induced cytotoxicity was conducted by combining the JPT and CHB (Asian population: ASN). Phased genotype data of the 186 ASN cell lines sequenced in the 1000 Genomes Project phase 1 version 3 (20) were downloaded from the BEAGLE website (http://faculty.washington.edu/browning/beagle/beagle.html). An additional 15 ASN samples genotyped in HapMap r28 were imputed to 1000 Genomes using BEAGLE version 3.3.2 (21). Phased genotype data were also downloaded for the 85 CEU and 88 YRI sequenced in the 1000 Genomes Project and used to impute the additional 93 CEU and 90 YRI samples genotyped in HapMap r28 with BEAGLE, which considers the relatedness of the trios in the imputation. Within each population, approximately 4.6 million SNPs [imputation $R^2 > 0.8$, population minor allele frequency (MAF) > 0.05, and in Hardy–Weinberg equilibrium ($P > 1 \times 10^{-6}$)] were tested for association with paclitaxel cytotoxicity using the linear mixed model Wald test, which accounts for the genetic relatedness of individuals, implemented in GEMMA version 0.94 (22).

Patient samples and GWAS

Germline DNA of 183 cancer patients treated with monotherapy paclitaxel stored in Biobank Japan (University of Tokyo) were genotyped using Illumina OmniExpress BeadChip that contained 733,202 SNPs (23). Of them, 24 patients experienced either grade 2 or higher paclitaxel-induced sensory peripheral neuropathy (PIPN), 121 patients revealed no neuropathy and the remaining 38 individuals had grade 1 neuropathy. For GWAS, we used 24 patients who developed ≥ grade 2 PIPN (cases), and compared them with 121 patients who did not show neuropathy (controls). The patient characteristics are described in Supplemental Table S1. The grade of toxicity was classified in accordance with the US National Cancer Institute's Common Toxicity Criteria version 2.0. All participants provided written informed consent. This project was approved by the Institutional Review Board in the Institute of Medical Science, the University of Tokyo, and RIKEN Center for Genomic Medicine. Sample quality control was carried out by methods including identity-by-state to evaluate cryptic relatedness for each sample, and population stratification by the use of principal component (PC) analysis to exclude genetically heterogeneous samples from further analysis. Then, our standard SNP quality control was carried out by excluding SNPs deviating from the Hardy–Weinberg equilibrium ($P \leq 10^{-6}$), non-polymorphic SNPs, SNPs with a call rate of < 0.99, and those on the X chromosome. We performed a genome-wide case/control allelic association study using Fisher's exact test.

LCL/Patient comparison

We used the SCAN database to determine which of the top SNPs significantly associated with paclitaxel-induced cytotoxicity in ASN ($P < 10^{-6}$) were located in or near (within 2 kb)

gene transcripts (24, 25). We also used the eQTL Browser (http://eqtl.uchicago.edu/Home.html) and GTEx Portal (http://www.gtexportal.org/home/) to detect whether SNPs genotyped in 1000 Genomes were eQTLs in LCLs and other tissues. The previous genomic studies using LCLs showed that Japanese and Han Chinese have similar genetic structure, but cluster separately from European and African-American individuals in principal components analysis (26). We used Sherlock, the computational algorithm that matches patterns of GWAS results with independent eQTL data to detect significant genes associated with the cytotoxicity and neuropathy phenotypes (18). We used the public HapMap CEU eQTL dataset (27) contained in Sherlock for gene association testing. Among the genes associated with each phenotype ($P < 0.05$) through Sherlock (18), the LCL/patient overlap genes were prioritized for functional analysis. Results were also compared with the published clinical GWAS of 855 genetic Europeans with breast cancer from CALGB40101, in which a dose-to-event analysis was conducted with an event defined as paclitaxel-induced sensory peripheral neuropathy ≥ grade 2 (9).

Functional studies in iPSC derived neurons

Human iPSC-derived cortical neurons, termed iCell Neurons (Cellular Dynamics International, Madison, WI, USA), were used for functional validation of the candidate genes. These cells have been previously used to model chemotherapy-induced neuropathy (28, 29).

RNA-Seq—RNA isolation from iPSC derived cortical neurons was performed after removal of media and addition of 50μL Qiazol per well plate for 5 minutes at room temperature to lyse cells. After vigorously pipetting each well several times, samples were collected and stored for further processing at -70°C. RNA was purified using the Ambion RNA protocol (15596026.PPS), only substituting Qiazol as the lysing reagent after consulting with the company. The final pellets were resuspended in 50 μl RNAase-free water dissolved at 55°C for 10 minutes, aliquoted and stored at -70°C. Nucleic acids quantification was performed using the Qubit dye for RNA kits (Molecular Probes, Life Technologies), as per manufacturer's specifications. 1 μg RNA from each time-point was submitted to the University of Chicago Genomics Core. RNA quality was checked on the Agilent Bio-analyzer 2100. RNA-SEQ libraries were generated in the core using Illumina RS-122-2101 TruSeq® Stranded mRNA LT Libraries and the final libraries checked again on the Agilent bio-analyzer 2100 which was followed by sequencing on the Illumina HiSeq2500. The raw sequencing reads were processed and analyzed using the RNA-Seq pipeline in the Galaxy Project (30). Briefly, the raw reads were mapped to the human genome reference (hg19) using TopHat2 (version 2.06) (31), which aligns RNA-Seq reads to the human genomes using the ultra high-throughput short read aligner Bowtie2 (32). Cufflinks (version 0.0.7) (33) was then used to quantify and quantile normalize the expression levels of the assembled transcripts.

siRNA knockdown studies—iCell Neurons (1.33×10^4) were seeded with 3.3μg/mL laminin (Sigma-Aldrich) in iCell Neuron complete maintenance media onto poly-D-lysine coated 96-well Greiner Bio-One plates (Monroe, NC, USA) according to manufactures recommendations. Dharmacon Accell technology (GE Dharmacon, Lafayette, CO, USA)

was applied at 4 hours post plating using 1 μM each human si*AIPL1, siPTPMT1*, siBCR or siDDX54 SMARTpool or the non-targeting control (NTC) and then 24 hours later cells were either collected for real time reverse transcription polymerase chain reaction (RT-PCR) or an exchange of transfection media with paclitaxel at a dose range of 0.001-10 μM or 0.17% DMSO vehicle control. After 24 or 48 hours, the drug was removed and cells were stained with 1 μg/mL Hoechst 33342 (Sigma-Aldrich) and 2 μg/mL Calcein AM in dPBS (Molecular Probes, Life Technologies Inc.; Carlsbad, CA, USA). Image analysis was performed on the ImageXpress Micro imaging system (Molecular Devices, LLC, Sunnyvale, CA, USA) at the University of Chicago Cellular Screening Center. Individual cell measurements of neurite outgrowth including mean/median/maximum process length, total neurite outgrowth (sum of the length of all processes), number of processes, number of branches, cell body area, mean outgrowth intensity and straightness were analyzed using the MetaXpress software Neurite Outgrowth Application Module. Each experiment was replicated three times and the difference in the relative neurite outgrowth for each siRNA treatment was compared to NTC and tested for significance by two-way ANOVA analysis.

Cells for RT-PCR from 2 wells for each experiment were collected at 24 and 48 hours post-transfection using Cells to CT (Life Technologies) as recommended by manufacturer. Two independent reverse transcription reactions were performed and the efficiency of knockdown for *AIPL1* (Hs04185077_m1), *PTPMT1* (Hs00378514_m1), BCR (Hs01036532_m1) or DDX54 (Hs_00225320_m1) (Life Technologies) for triplicate wells was determined using Taqman fast chemistry on the Viia7 PCR machine (LifeTechnologies). A comparative delta delta CT analysis was used to determine the percent knockdown of each gene by comparing the CT difference relative to the beta-2-microglobulin control (NM_004048.2) and normalizing to NTC.

Results

Paclitaxel-induced cytotoxicity in LCLs

HapMap LCLs were treated with increasing concentrations of paclitaxel and cellular growth inhibition was measured. The $\log_2$-transformed area under the survival curve (AUC) was used as the paclitaxel-induced cytotoxicity phenotype. To evaluate ethnic differences in mean $\log_2$ AUC of paclitaxel, we compared results from LCLs derived from the Asian populations (JPT, n = 57; CHB, n = 59) to our previous data of three other HapMap LCL populations (CEU, n = 77; YRI, n = 87; ASW, n = 83) (19). The paclitaxel-induced $\log_2$-transformed cytotoxicity phenotype showed normal distributions in each population. Figure 1 illustrates the paclitaxel-induced cytotoxicity among 5 populations with no statistical differences among the populations observed.

Genome-wide association study in Asian LCLs

Figure 2 illustrates the Manhattan plot of paclitaxel-induced cytotoxicity in LCLs derived from the Asian populations (JPT, CHB). There were 248 SNPs associated with paclitaxel cytotoxicity in the ASN population at a suggestive significance of $P < 10^{-5}$; however among them only 1 SNP (rs56763443) and 2 SNPs (rs8049617 and rs7598550) were significant in the CEU and YRI GWAS results at $P < 0.05$, respectively. Three of the most significant 6

SNPs in the ASN GWAS of paclitaxel-induced cytotoxicity were located near (within 2 kb) gene *AIPL1* (aryl-hydrocarbon-interacting protein-like 1). The three AIPL1 SNPs are in perfect LD (r^2=1, D'=1 in ASN) and thus represent one signal. We evaluated whether any of these 6 SNPs were expression quantitative trait loci eQTLs with $r^2 \geq 0.8$ in two distinct databases, University of Chicago database (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/) and GTEx Portal (http://www.gtexportal.org); however they were not eQTLs in any tissues listed (generic genome browser version 1.68). We also downloaded the expression data collected from ASN LCLs in Stranger et al. (34). We found no relationship between *AIPL1* expression and paclitaxel cytotoxicity (n = 88, β = -0.012, P = 0.66) or *AIPL1* expression and rs3892315 genotype (n = 90, β = -0.009, P = 0.52). Table 1 illustrates these 3 SNPs trending toward significance in LCLs derived from individuals of Northern/Western Europe (CEU; $P < 0.065$-0.078) but there was no significance in African LCLs (YRI; p = 0.305-0.355). Thus, the genetic variation near *AIPL1* may contribute to paclitaxel-induced cytotoxicity particularly in Asians. Supplemental Table S2 lists 28 SNPs in or near *AIPL1* and their association with sensory neuropathy in Asian patients; one SNP (rs7214517) has a p-value of 0.028. The 3 LCL SNPs (rs56763443, rs8049617 and rs7598550) were not genotyped in the Asian study.

Functional validation of *AIPL1* using human iPSC-derived neurons

The effects of *AIPL1* expression on paclitaxel-induced neurotoxicity was assessed in neurons derived from induced pluripotent stem cells (iPSC). To evaluate the effects of low *AIPL1*, expression on paclitaxel-induced neurotoxicity, we modulated the gene in neurons through transient siRNA transfection and verified with quantitative polymerase chain reaction (qPCR), a reduction of expression in AIPL1 to 62% and 14% relative to control at 24 and 48 hours post transfection relative to non-targeting control (Figure 3A). The modulation of expression of *AIPL1* resulted in increased resistance of neurons to paclitaxel at 48 hours using two-way ANOVA analysis, as measured by significant increase in relative total outgrowth (Fig. 3B; $P = 0.017$), mean process length (Fig. 3C; $P = 0.008$) and relative number of processes (Fig. 3D; $P = 0.029$). Median process length ($P = 0.005$), max process length ($P = 0.011$), phenotypes related to mean process length and mean outgrowth intensity, a phenotype related to thickening of the neurite outgrowths (P= 0.0065) were also significantly different (data not shown). Figure 3E illustrates morphological changes for NTC and siAIPL1 treated cells for neurite outgrowth following 48 hours paclitaxel treatment of iPSC-derived neurons by high content imaging.

GWAS in patient samples of paclitaxel induced neuropathy

We performed GWAS of paclitaxel-induced neuropathy in 183 Asian cancer patients (Figure 4A). We identified 4 SNPs above a suggestive threshold of $p<10^{-5}$ and no genome wide significant SNPs at $p = 5\times10^{-8}$. Gene based tests are commonly used following traditional SNP based analysis because they reduce multiple testing and provide genes as opposed to SNPs. Therefore, we evaluated LCL Asian GWAS (Figure 2), Asian cancer patient data (Figure 4A) and GWAS data from CALGB40101, a study of 855 Europeans evaluated for paclitaxel-induced neuropathy (9) using Sherlock, a program that enables gene-based sorting of targets by scoring alignment of genetic signatures of eQTLs and the phenotype (18). Comparison of results from Asian LCL cytotoxicity and Asian patient

neuropathy at $P < 0.05$, had 32 overlap genes (Fig. 4B, Supplemental Table S3); comparison of Asian LCL cytotoxicity and European patient neuropathy at $P < 0.05$ had 37 overlap genes (Fig. 4B, Supplemental Table S4) and comparison of Asian and European patient GWAS cohorts at $P < 0.05$ had 10 overlap genes (Fig. 4B, Supplemental Table S5). Interestingly, *PTPMT1* (protein tyrosine phosphatase, mitochondrial 1) overlapped all 3 analysis: Asian LCL cytotoxicity, Asian patient neuropathy, and European patient neuropathy at $P < 0.05$. Although we modulated gene expression of *PTPMT1* in iCell neurons, resulting in 77 and 41% gene remaining after 24 and 48 hours, no significant difference was found in any of the neurite outgrowth phenotypes when compared to NTC (data not shown), indicating that some mechanism distinct from levels of gene expression may explain the role of *PTPMT1* in paclitaxel-induced peripheral neuropathy. Alternatively, the effect of gene knockdown on other neuronal phenotypes such as electrical activity would not be captured in this system that is focused on morphological changes.

Functional validation of *BCR* and *DDX54* using human iPSC-derived neurons

To prioritize the 32 Asian specific genes (Supplemental Table S3), we evaluated both their gene level expression in iPSC derived neurons (using RNA-Seq) and published literature using Pubmed search terms: gene name and CIPN or neuropathy or neurons done 2/2015 (Supplemental Figure 6). Breakpoint cluster region (Bcr) protein, highly expressed in iPSC derived neurons, is enriched in neurons and involved in neural activities and therefore was chosen for further studies (35-37). We found that a change in expression to 62% and 67% at 24 and 48 hours compared to NTC (Fig 5A), resulting in a decrease in the relative total outgrowth ($P = 0.0097$), number of processes ($P = 0.0298$), max process length ($P = 0.0016$) and number of branches ($P = 0.0003$) (Figs. 5B-D) after treatment with paclitaxel in three independent experiments. Ddx54 protein has been shown to play a critical role in central nervous system myelination, presumably in myelin sheath formation after the differentiation of oligodendrocytes (38). DDX54 (DEAD-box RNA helicase) was modulated to 66% and 61% at 24 and 48 hours, but resulted in no significant changes in any neurite phenotype measured including relative total outgrowth, number of processes, max process length and number of branches.

Discussion

A comparison of paclitaxel-induced cytotoxicity in LCLs derived from patients of Asian, European and African ancestry demonstrates no difference in cellular sensitivity to paclitaxel. Traditional GWAS reveals only a small fraction (3 out of 248) SNPs suggestively significant in Asian LCLs for paclitaxel induced cytotoxicity are also significant in either CEU or YRI LCLs with the most significant genetic variant associations unique within populations. The top intragenic SNPs in the ASN GWAS of paclitaxel-induced cytotoxicity were located near *AIPL1* gene. Upon knockdown of *AIPL1* in human induced pluripotent stem cell derived neurons, decreased sensitivity to paclitaxel as measured by neurite outgrowth phenotypes. Using a gene based approach, a gene identified as Asian specific (overlapped in Asian LCLs and Asian clinical cohort of neuropathy) *BCR* (breakpoint cluster region), was validated using iPSC-derived neurons with knockdown corresponding to increased sensitivity to paclitaxel induced damage to neurons. The same approach identified

PTPMT1 associated with paclitaxel-induced cytotoxicity in Asian LCLs, as well as Asian and European paclitaxel induced peripheral neuropathy clinical cohorts. By integrating gene-based analyses from clinical studies of paclitaxel-induced neuropathy in Asian and European patients with LCL results, population specific and across population target genes were identified.

The HapMap LCLs are advantageous for evaluating pharmacogenomics of anticancer agents because these samples have publicly available genotypes (39, 40) and sequencing data (20) for genome wide association studies. The samples also have gene expression (41), miRNA (42), modified cytosine (43) and protein data (44) that allow for the elucidation of potential function of the genetic variants associated with pharmacologic phenotypes. Our observation of a lack of population difference in paclitaxel-induced cytotoxicity is in contrast to population differences observed for platinating agents (45), and antimetabolites (46). In part, these population differences in drug sensitivity may be explained by population differences in gene expression (41). The role of gene expression in population differences in chemotherapeutic cytotoxicity is supported by the observation that pharmacologic SNPs for several different chemotherapeutic agents (etoposide, daunorubicin, cisplatin, carboplatin, cytarabine, capecitabine) are enriched in eQTLs or SNPs associated with gene expression (25). In contrast, pharmacologic SNPs associated with paclitaxel are not enriched in eQTLs; however enrichment in protein quantitative trait loci has been observed (47). These differences may be due to differences in mechanism of action of paclitaxel, a microtubule stabilizer compared to the other cytotoxic agents that target DNA.

In our Asian patient dataset, it appears that peripheral neuropathy is more common in females than males (males: 1 case/33 controls; females: 23 cases/88 controls); however, this gender difference is likely confounded by differences in cumulative dose, treatment schedule or age of the patients. Evidence suggests that age, race, and comorbid conditions, such as diabetes, and cumulative dose of drug administered may be associated with an increased risk of developing neuropathy (48).

In efforts to decrease multiple tests and identify genes instead of SNPs, we chose to utilize a gene-based approach to detect clinically significant genes with multiple trans eQTLs with moderate associations, which cannot be identified from the GWAS alone (18). We employed Sherlock to compare genes significant in two patient studies of paclitaxel induced neuropathy (855 European and 183 Japanese) and the LCL data we generated in the Asian populations. According to the gene-based test, 10 genes including *PTPMT1* were found to overlap between Asians and Europeans along with hundreds of candidate gene associations that were unique to each population. At this threshold, *PTPMT1* was revealed as a common target of paclitaxel-induced sensory peripheral neuropathy among populations. PTPMT1 is anchored in the inner mitochondrial membrane and is required for mitochondrial integrity and oxidative respiration (49). Because its ablation increased apoptosis in cancer cell lines and decreased differentiation and proliferation, but not survival in embryonic stem cells, its function may be cell-type specific in the regulation of cell growth and survival (50). Thus *PTPMT1* in peripheral nerve mitochondria may play a critical role in paclitaxel-induced neurotoxicity across populations and is worth further studies. Our data indicates expression knockdown *PTPMT1* in iPSC derived neurons did not alter sensitivity to paclitaxel as

measured by morphological changes in neuronal outgrowths; however it is possible that the gene is important in electrical activity or other measures of neuronal damage.

Through LCL GWAS of paclitaxel-induced cytotoxicity, SNPs in *AIPL1*, were implicated as the most significant intragenic SNPs in the Asian LCLs. In evaluating SNPs within or close to *AIPL1*, rs7214517 was associated with Asian patient peripheral neuropathy ($p = 0.028$). *AIPL1* expressed in rod and cone photoreceptors has a critical role in cell viability, and gene defects in *AIPL1* cause heterogeneous degenerations of the human retina, such as retinitis pigmentosa (51). Interestingly, this disease is related to NARP syndrome (neuropathy, ataxia and retinitis pigmentosa) which is an inherited condition chiefly affecting nervous systems due to mitochondrial DNA dysfunction in creating adenosine triphosphate (52). Its worldwide prevalence is unknown and it remains unclear how this disruption in mitochondrial energy production leads to muscle weakness, vision loss and the other specific features of NARP syndrome. Kirschman et al. also found that transgenic expression of *AIPL1* restored rod morphology and the rod-derived electroretinogram response in $Aipl^{-/-}$ mice, implicating a neuron component for this gene (53). Mitochondrial dysfunction has been implicated as a mechanism for neurotoxicity of paclitaxel (54) and our results demonstrated that decreased expression of *AIPL1* significantly decreased sensitivity of neurons to paclitaxel, hence, there could be a cell-type specific function of AIPL1 in a mitochondrial component in peripheral neurons and *AIPL1* could be a potential therapeutic target of paclitaxel-induced sensory peripheral neuropathy especially in Asians.

We identified a set of 32 Asian specific genes that overlapped in the Asian LCL and Asian patient datasets. One of those genes, *BCR*, was functionally validated in the iPSC derived neuronal model with increased sensitivity of neurons to paclitaxel upon knockdown of the gene. *Bcr* has been shown in rat PC12 cells to cooperate with Fes to activate Rho family GTPases as part of a novel pathway regulating neurite extension (55). Furthermore, Huntington's-associated protein, 1A interacts with Bcr on microtubules to regulate neuronal differentiation (56). Increasingly, Bcr is emerging as an important regulator of excitatory synapse receptor expression and development. Our data indicates that BCR contributes to the protection of neurons from paclitaxel induced damage as measured by several neuronal phenotypes including total outgrowth of neurites.

Although CIPN is a common, potentially severe and dose-limiting adverse effect of many commonly used cancer therapeutics including paclitaxel, there are no effective genetic markers or treatment for this side effect (4, 7, 8). Therefore, to identify genetic variants and genes associated with CIPN, several large clinical GWAS have been conducted and revealed a few promising associations (9, 10, 14). However, there are some considerable issues in clinical GWAS of CIPN including: 1) lack of replication studies; 2) pharmacogenomics underlying ethnic specificity and; 3) lack of appropriate models to functionally validate important GWAS results. Therefore, combining cell-based data with multiple clinical genome wide association studies, as done in this study, is a way to identify the most promising genes. Furthermore, we utilized a novel technology, human iPSC-derived neurons for gene functional validation. The elucidation of variants specific to the Asian population may be helpful in developing personalized cancer treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance

Chemotherapy-induced peripheral neuropathy (CIPN) causes a significant symptom burden because many patients that undergo adjuvant treatment are long-term survivors. Moreover, CIPN can lead to dose reduction of the chemotherapeutic agent or possible cessation of treatment. This may have an adverse impact on cancer treatment and disease outcomes. In this study, we have used a genome wide approach in a clinical and preclinical setting to identify genetic variants associated with peripheral neuropathy in Asians. The findings can be used in a clinical setting to dose reduce patients at risk or alter therapy to avoid this devastating side effect. In addition, an improved understanding of the pathophysiology of paclitaxel-induced neuropathy in and across populations will assist in the development of future neuroprotective agents and in the design of novel therapies with improved toxicity profiles.

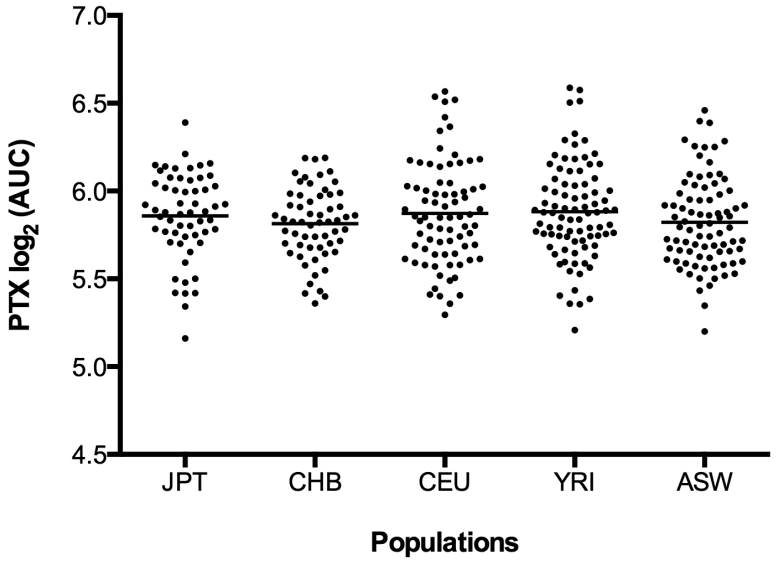

Figure 1. Cellular sensitivity to paclitaxel in 363 HapMap LCLs

LCLs from different world populations (JPT: n = 57, CHB: n = 59, CEU: n = 77, YRI: n = 87, ASW: n = 83) were phenotyped for the cellular growth inhibitory effects of paclitaxel. The cytotoxicity phenotype is $\log_2$-transformed mean area under the paclitaxel concentration-cell survival percentage curve (AUC). There were no statistically significant differences among the 5 populations.

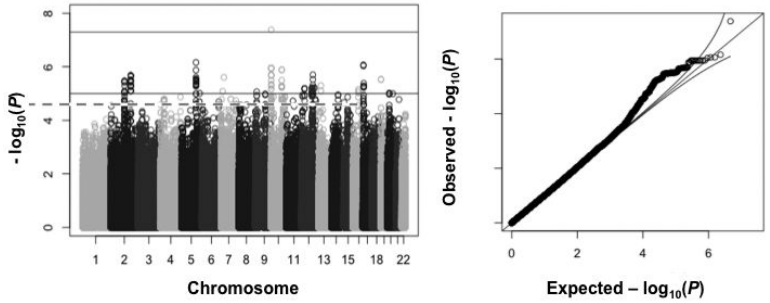

Figure 2. Asian LCL GWAS results

Paclitaxel-induced cytotoxicity in Asian LCLs. Top line in Manhattan plot is at the genome-wide significance threshold of $P = 5\times10^{-8}$ and below line is at the suggestive significance threshold of $P = 10^{-5}$. Q-Q plots show some signal above the expected p-values (black lines represent the expected 95% confidence intervals) in the LCL GWAS.

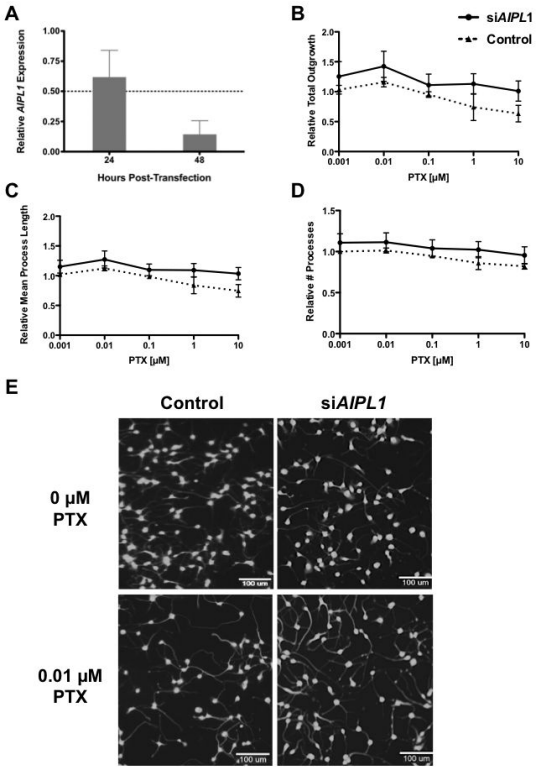

Figure 3. Decreased *AIPL1* expression reduces sensitivity of human iPSC-derived neurons to paclitaxel

A) Relative *AIPL1* expression 24 to 48 h post-transfection normalized to NTC. Neurite outgrowth phenotypes measured following 48 h paclitaxel exposure (0.001-10 μM) were tested for differences between si*AIPL1* and NTC by two-way ANOVA including B) relative total neurite outgrowth ($P = 0.017$), C) relative mean process length ($P = 0.008$), and D) relative number of processes ($P = 0.029$). Error bars represent the standard error of the mean from three experiments of >2000 cells per dose. E) Representative high content images comparing human iPSC-derived neurons upon siRNA knockdown of *AIPL1* and non-targeting control (NTC) 48 h post-treatment with 0.01 M paclitaxel. Images taken at 10× magnification, scale bar = 100 μm.

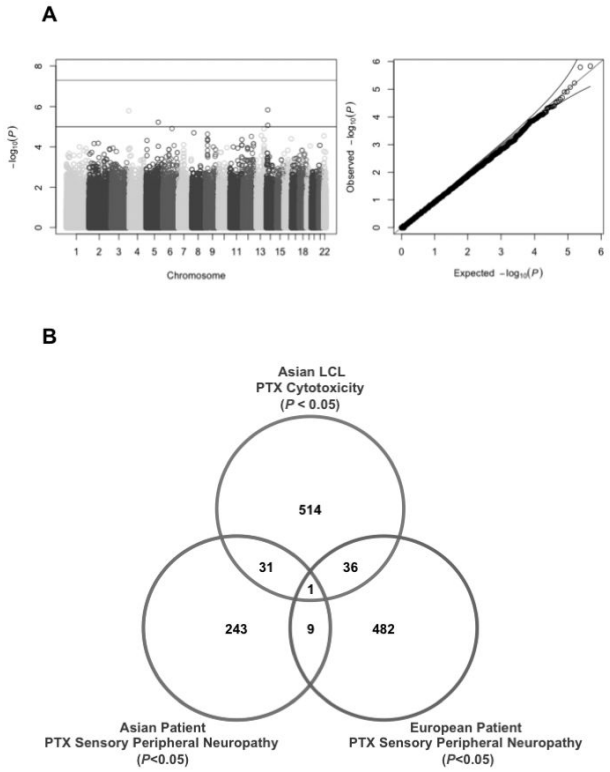

Figure 4. Asian patient GWAS results and LCL/patient overlap genes

A) Paclitaxel-induced sensory peripheral neuropathy in Asian patients. Top line in Manhattan plot is at the genome-wide significance threshold of $P = 5\times10^{-8}$ and below line is at the suggestive significance threshold of $P = 10^{-5}$. Q-Q plots show no signal above the expected p-values (black lines represent the expected 95% confidence intervals) in the patient GWAS. B) LCL/patient overlap genes associated with paclitaxel-induced phenotypes ($P < 0.05$, number: genes). Thirty-two genes were overlapped between Asian LCL cytotoxicity and Asian patient neuropathy ($P < 0.05$). PTPMT1 surpassed the threshold for association in all 3 analyses.

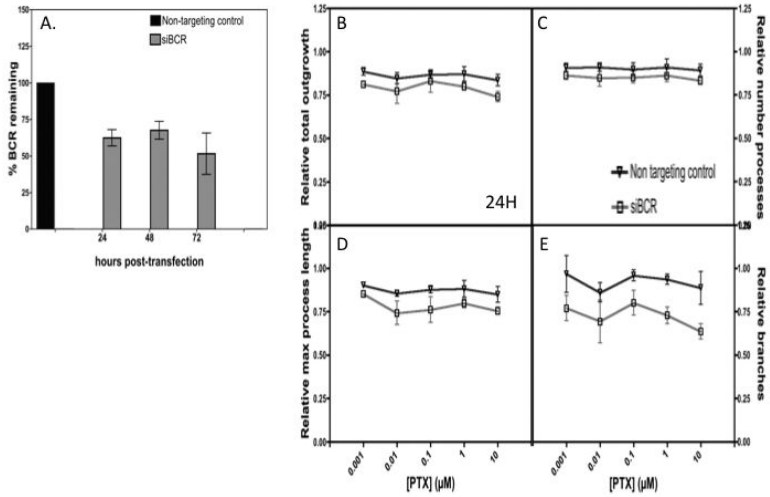

Figure 5. Decreased *BCR* expression reduces sensitivity of human iPSC-derived neurons to paclitaxel

A) Relative *BCR* expression 24h post-transfection normalized to NTC. Neurite outgrowth phenotypes measured following 24 h paclitaxel exposure (0.001-10 μM) were tested for differences between si*BCR* and NTC by two-way ANOVA including relative B) total neurite outgrowth ($P = 0.0097$), C) number of processes ($P = 0.0298$) D) max process length ($P = 0.0016$), and E) number of branches ($P = 0.0003$). Error bars represent the standard error of the mean from three experiments of >2000 cells per dose.

Table 1

SNPs significantly associated with paclitaxel-induced cytotoxicity in Asian LCLs ($P < 10^{-6}$) and their level of significance in CEU LCLs and YRI LCLs.

	ASN			CEU			YRI			
SNP	Beta	*P* value	MAF	Beta	*P* value	MAF	Beta	*P* value	MAF	Host Gene
rs11595500	−0.212	4.13E-08	0.167	−0.036	0.463	0.346	0.171	0.149	0.058	NA
rs264124	0.146	6.95E-07	0.343	0.020	0.694	0.463	-	-	-	NA
rs3892315	0.207	8.72E-07	0.134	0.151	0.065	0.113	−0.047	0.353	0.321	*AIPL1*
rs11651916	0.207	8.75E-07	0.134	0.144	0.078	0.113	−0.050	0.307	0.317	*AIPL1*
rs3892316	0.206	9.23E-07	0.137	0.151	0.066	0.113	−0.047	0.355	0.321	*AIPL1*