Clinical Cancer Research

SK3 Gene Polymorphism Is Associated with Taxane Neurotoxicity and Cell Calcium Homeostasis

Carina Rua[1,2,3], Maxime Guéguinou[1,3], Imane Soubai[2,4], Erika Viel[2], Marie Potier-Cartereau[1,3,5], Aurélie Chantome[1,3], Catherine Barbe[2], Philippe Bougnoux[1,2,3,6], Chantal Barin-Le Guellec[6,7], and Christophe Vandier[1,3,5]

Abstract

Purpose: Taxane-induced peripheral neuropathy is a common side effect induced by anticancer agents, and no drug capable of preventing its occurrence or ameliorating its long-term course has been identified. The physiology of taxane neuropathy is not clear, and diverse mechanisms have been suggested, with ion channels regulating Ca^{2+} homeostasis appearing good candidates. The calcium-activated potassium channel SK3 is encoded by the *KCNN3* gene, which is characterized by a length polymorphism due to variable number of CAG repeats.

Experimental Design: To study the influence of the polymorphism of CAG motif repeat of *KCNN3* on the development of taxane-induced neuropathy, we evaluated 176 patients treated with taxanes for breast cancer. In parallel, we measured Ca^{2+} entry using Fura2-AM dye in HEK cells expressing short versus long CAG alleles of *KCNN3*.

Results: In the current study, we report that in the presence of docetaxel, Ca^{2+} entry was significantly increased in cells expressing short versus long CAG alleles of SK3 and that a SK3-lipid blocker inhibits this effect. We found that patients carrying a short *KCNN3* allele exhibited significantly increased incidence of taxane neuropathy compared with those carrying longer alleles.

Conclusions: The clinical implication of these findings is that *KCNN3* polymorphism may increase patient susceptibility to taxane neurotoxicity and that the use of SK3 blockers during taxanes' administration may represent an interesting approach for the prevention of this neurotoxicity. *Clin Cancer Res; 24(21); 5313–20. ©2018 AACR.*

Introduction

Taxane-induced peripheral neuropathy is a common side effect induced by paclitaxel and docetaxel (1, 2). In clinical practice, the most common feature of taxanes neurotoxicity is a predominant sensory distal neuropathy with paresthesias, dysesthesias, burning dysesthesia, numbness, tingling, and shooting pains (2). These side effects may seriously compromise the quality-of-life of patients and taxane schedule modifications are often required to limit their severities, which could potentially prevent patients from receiving an effective cancer treatment. To our knowledge, no drug capable of preventing the occurrence of taxane-induced peripheral neuropathy or ameliorating its long-term course has been identified. It is therefore critical to understand the mechanisms regulating taxane neurotoxicity to develop new strategies that limit or prevent peripheral neuropathy. Several hypotheses have been postulated including different taxane metabolisms between patients resulting in an increased drug exposure for some patients or the existence of a direct drug toxicity on peripheral neurons. Paclitaxel was actually found to cause degeneration of peripheral and central branches of dorsal root ganglia axons (3), and this effect may be mediated by the activation of the Ca^{2+}-activated proteases calpains (4). Because Ca^{2+} concentration is regulated by ion channels, another hypothesis is that taxanes neurotoxicity may involve ion channels regulating Ca^{2+} homeostasis. Paclitaxel (Taxol^{R}) was also found to activate the transient receptor potential channel (TRPV4; ref. 5), and paclitaxel can activate the voltage gated Ca^{2+} channel CaV3.2 (6). Docetaxel was also found to modulate the activity of K^{+} channels known to regulate membrane potential and therefore Ca^{2+} entry (7). Interestingly, the SK3 potassium channel has been found to be associated with oxaliplatin neurotoxicity (8), and we reported a pivotal role of this channel in the regulation of Ca^{2+} homeostasis and Ca^{2+}-activated proteases calpains (9, 10). SK3 channel belongs to Ca^{2+}-activated K^{+} channel family (K_{Ca}). K_{Ca} channels can be divided into three subfamilies: large or big conductance K_{Ca} (BK_{Ca}), intermediate conductance K_{Ca} (IK_{Ca}), and small conductance K_{Ca} (SK_{Ca}). SK_{Ca} channels are voltage insensitive and are activated by low concentrations of cytosolic Ca^{2+}. There are three isoforms of SK_{Ca} alpha subunits, named SK1, SK2, and SK3, which associate to form homo- or heterotetramers (11).

[1]Université de Tours, Inserm, N2C UMR1069, Tours, France. [2]CHRU Bretonneau, Tours, France. [3]Ion channels and Cancer Network of Canceropole Grand Ouest (IC-CGO), Nantes, France. [4]Université de Tours, Faculté de Pharmacie, Tours, France. [5]Université de Tours, Faculté de Sciences et Techniques, Tours, France. [6]Université de Tours, Faculté de Médecine, Tours, France. [7]Université de Limoges, Inserm, UMR1248, Limoges, France.

C. Rua and M. Guéguinou contributed equally to this article.

Current address for E. Viel: Ramsay Générale de Santé, Hôpital Privé Sainte-Marie, Chalon/Saône, France and Institut de Cancérologie de Bourgogne, Chalon/Saône, France; and current address for M. Guéguinou, Université de Tours, GICC EA 7501, Tours, France.

Corresponding Author: Christophe Vandier, Inserm, UMR1069, 10 Boulevard Tonnelle, Tours 37032, France. Phone: 332-4736-6024; Fax: 332-4736-6226; E-mail: christophe.vandier@univ-tours.fr

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Translational Relevance

Taxane-induced peripheral neuropathy is a common side effect induced by anticancer agents, but its physiopathology remains unclear. Until now, no drug capable of preventing its occurrence or ameliorating its long-term course has been identified. The current study demonstrates for the first time the influence of CAG repeat length of the SK3 channel in the control of calcium entry in the presence of docetaxel and in the incidence of taxane neuropathy. With these results, we inform the scientific community that *KCNN3* polymorphism may correlate with taxane neurotoxicity in patients. An implication of this finding is the possibility provided to oncologists to address taxane neurotoxicity by altering the activity of SK3 channel when SK3 blockers become clinically available. In that regard, we currently propose the use of our recently developed alkyl-ether lipids, of which certain belong to a new class of SK3 blockers, as inhibitors of taxane neurotoxicity.

Contrary to SK1, SK2, and SK3 channels are not restricted to neuronal tissues (12), and SK3 channels were found to be expressed in dorsal root ganglion neurons, where they are activated after sensory stimulation and act to control sensory input into the spinal cord (13).

In excitable cells such as neurons, the activation of SK3 channels by low Ca^{2+} concentrations, results in hyperpolarization of the plasma membrane and changes in cellular excitability. In neurons, SK3 channel activation also counteracts further increases in cytosolic Ca^{2+}, thereby regulating its concentration and controlling neuronal firing properties and neurotransmitter release (14). The *KCNN3* gene is highly polymorphic with the presence of a variable number of CAG triplet repeats in the region encoding the N-terminal domain. It was demonstrated that cells carrying alleles with an elevated number of CAG repeats (i.e., long length alleles containing 24 CAG repeats compared with 18 or 11) display reduced SK3 channel activity with a reduction of the amplitude of SK3 current and a left-shift of the inward rectification to reduced membrane potential values (15). Previous studies have demonstrated that SK3 channel regulates neuronal activity through the regulation of after hyperpolarization (16), and the length of the CAG repeat could generate different after hyperpolarization, thereby modulating neuronal excitability.

Interestingly, a relationship between *KCNN3* polymorphism and the severity of peripheral neuropathy following oxaliplatin treatment has been identified in colon cancer, with patients that carry short CAG repeats (14–16) experiencing more severe neuropathy (8).

In the current study, we tested the effect of docetaxel on the regulation of cytosolic Ca^{2+} concentration that is controlled by the SK3 channel activity in cells carrying long and short CAG repeats in *KCNN3*. To study the influence of the polymorphism of CAG motif repeat of *KCNN3* on the development of taxane-induced neuropathy, we evaluated patients treated with taxanes for breast cancer.

Materials and Methods

In vitro analysis

HEK293 cells were maintained in DMEM, supplemented with 10% (v:v) FBS (Lonza) and were grown in a humidified atmosphere at 37°C (95% air, 5% CO_2). HEK293 cells, expressing short (11 CAG repetitions) or long (24 CAG repetitions), SK3 channel isoforms were kindly provided by L. Pardo (Max Planck Institute of Experimental Medicine, Göttingen, Germany). Genetic analysis of *KCNN3* polymorphism was controlled as described below. Three days after thawing and 15 days later, cells were monitored for *Mycoplasma* contamination using the MycoAlert Mycoplasma Detection Kit (Lonza) and were replaced after one month in culture. Number passages were limited to 13 after stable transfection with SK3 channel isoforms.

Drugs and solutions

Drugs were added to the physiologic saline solution (PSS) or culture media at the concentrations indicated in figure legends. All drugs were purchased from Sigma-Aldrich.

The PSS or "2Ca" solution had the following composition (in mmol/L): NaCl 140, $MgCl_2$ 1, KCl 4, $CaCl_2$ 2, D-glucose 11.1, and HEPES 10, adjusted to pH 7.4 with NaOH. The Ca^{2+} free solution or "0 Ca" is a PSS solution without $CaCl_2$ and with 1 mmol/L EGTA.

Ca^{2+} entry measurements

The effect of docetaxel on cytosolic Ca^{2+} was evaluated and compared using HEK293 cells that expressed different *KCNN3* CAG repeat length [SK3$(CAG)_{24}$ and SK3$(CAG)_{11}$]. Time-lapse [Ca^{2+}] cytosolic measurements were performed using the FlexStation III fluorometer (Molecular Devices). HEK293 cells were seeded on 96-well flat clear-bottom black microplates (#3603, Corning) at a density of 3×10^4 cells/well. HEK293 cells were grown on fibronectin-coated microplates. One day after seeding, HEK293 cells were incubated with 4 μmol/L Fura-2/AM in culture medium for 35 minutes at 37°C protected from light. Afterward, the cells were gently washed three times with HEPES-buffered saline (140 mmol/L NaCl, 1.13 mmol/L $MgCl_2$, 4.7 mmol/L KCl, 2 mmol/L $CaCl_2$, 10 mmol/L D-glucose, and 10 mmol/L HEPES, pH 7.4) and left for 5 minutes at room temperature and protected from light. Data acquisition was performed using SoftMax pro software. Fura-2–loaded cells were alternately illuminated with 340 and 380 nm. Fluorescence emission was collected at 510 nm. Figures showing Ca^{2+} traces show averages from several well per condition and are representative of several independent recordings.

In HEK293 cells, Ca^{2+} homeostasis is regulated by store-operated channels that mediate store-operated Ca^{2+} entry (SOCE). An easy method to induce SOCE is to deplete Ca^{2+} from the endoplasmic reticulum using thapsigargin (a SERCas inhibitor). Immediately after the loading, cells were washed in PSS with Ca^{2+}. One minute before the run, we placed the cells in PSS-free Ca^{2+} solution and treated with thapsigargin (2 μmol/L; T7458, Life Technologies): After intracellular store depletion, 2 mmol/L $CaCl_2$ was injected (2 Ca solution). Fluorescence emission was measured at 510 nm with an excitation light at 340 and 380 nm (FlexStation 3).

Clinical study

Patients and protocol. This study was conducted between November 2012 and May 2014 in a population of women treated for breast cancer using taxanes at the University Hospital of Tours (Tours, France). The study was approved by the Ethics committee of the hospital, was conducted in accordance with recognized ethical guidelines, and written informed consent was obtained

from all patients. Patients with preexisting neuropathy or who had received chemotherapy known to induce neuropathy (vinca-alkaloids, platinum, eribulin mesylate, or bortezomib) were not eligible. Patients had never received taxanes before this study. Included patients received either 100 mg/m^2 docetaxel via intravenous infusion on day 1 of a 21-day cycle or 60 mg/m^2 paclitaxel as a weekly intravenous infusion every 21 or 28 days. The neoadjuvant and the adjuvant protocols lasted 4 and 3 cycles, respectively. The duration of the metastatic protocol depended on each patient tolerance.

Neurologic study. Depending on patient history and their physical examination, neuropathies were evaluated using the NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE). The grade of neuropathy was based on the duration and intensity of symptoms (grade 0: no symptoms; grade 1: paresthesia and/or decreased osteotendinous reflexes; grade 2: severe paresthesia and/or weak muscle weakness; grade 3: intolerable paresthesia and/or significant motor loss and grade 4: paralysis). The time, intensity, and doses of taxanes were recorded at the emergence of neuropathy, and the same data were collected if the patient demonstrated an increased grade during chemotherapy. During the follow-up, the continuation of the chemotherapy was recorded.

Genetic analysis of KCNN3 polymorphism. Genomic DNA was extracted from peripheral blood leukocytes using a FlexiGene DNA Kit according to the manufacturer's instructions (QIAGEN). The DNA region containing the repeated trinucleotide sequence (in exon 1) was amplified by the fluorescent PCR method with a DNA thermal cycler, using the sense primer 5′-CAGCAGCCCC-TGGGACCCTCGC and the antisense 5′ 6-FAM GGAGTTGGGC-GAGCTGAGACAG. PCR was carried out in a total volume of 50 µL containing 100 ng of genomic DNA, 1 µL of each primer (10 µmol/L), 5 µL Accuprime PCR buffer II (10×), and 1 µL of Taq polymerase (Invitrogen). The amplification protocol was as follows: denaturation at 95°C for 4 minutes, 22 thermocycling cycles (annealing at 55°C for 60 seconds and primer extension at 72°C for 30 seconds), and a final extension at 72°C for 10 minutes. The length of the PCR product generated depended on the number of CAG repeats. PCR products were separated by size using capillary electrophoresis on an Applied 3130XL automated DNA capillary sequencer (Applied Biosystems). GeneScan 500 ROX Size Standard was used as standard to extrapolate the size of the sample product peaks. Because both the position of the primers used in the PCR and the number of bases amplified that are not CAG repeats are known, it is easy to deduce the number of CAG repeats from the size of fragments obtained by capillary electrophoresis. Furthermore, the sizes of polymorphic amplicons should differ by a multiple of 3 units from each other. Using the reference sequence of *KCNN3* gene in the Ensembl gene browser (https://www.ensembl.org), the expected fragment size of the allele comprising 14 CAG repeats is 121 pb. However, to further validate our method and standardize our allele sizes, we compared it with direct sequencing. The DNA region containing the polymorphism was amplified using the same primers, albeit unlabeled for the antisense one, and the same PCR conditions except for the number of thermocycling cycles that was increased to 45. Electrophoretic Separation of DNA fragments was performed on agarose gels. After cutting the DNA bands out of the agarose gel, the DNA samples were purified and analyzed by Sanger chain termination DNA sequencing. The number of CAG repeats was determined by direct counting of these triplets of bases on electropherograms. The concordance between the two methods was assessed in 12 patients by plotting the number of CAG repeats counted on the electropherograms against the length of the fragments obtained from capillary electrophoresis. Once validated, this latter method was preferred for genotyping of the whole cohort as it is much easier to interpret as compared with electropherograms.

To explore a potential role of the *KCNN3* CAG repeat lengths, we used the sum of both alleles as described previously for schizophrenia (15). We use the median of the sum of CAG repeats distribution to determine each group.

Statistical analysis

Means with SD or percentage were used for descriptive data. We used the median to divide long versus short group in our cohort. χ^2 test was used to compare data. Differences between Kaplan–Meier survival curves for probability of neuropathy were tested by log-rank test. Analyses were conducted with GraphPad Prism 4. *N* indicates the number of experiments and *n* the number of samples.

Results

Effect of docetaxel on Ca^{2+} entry depending on *KCNN3* polymorphism

To determine whether *KCNN3* polymorphism was associated with a modulation of calcium metabolism in cells, HEK293 cells carrying SK3$(CAG)_{24}$ or SK3$(CAG)_{11}$ were obtained and tested. Figure 1A shows that thapsigargin can induce a transient increase of cytosolic Ca^{2+} in the absence of external Ca^{2+} followed by a large increase of cytosolic Ca^{2+} after addition of external Ca^{2+} (a.k.a., SOCE). This finding was observed with HEK293-SK3$(CAG)_{24}$ and HEK293-SK3$(CAG)_{11}$. The amplitude of SOCE was not significantly different between HEK293-SK3$(CAG)_{24}$ and HEK293-SK3$(CAG)_{11}$ cells under control condition (Fig. 1B). However, in the presence of 10 µmol/L docetaxel, the amplitude of SOCE was significantly increased in HEK293-SK3$(CAG)_{11}$ compared with HEK293-SK3$(CAG)_{24}$ cells (Fig. 1B). Interestingly, docetaxel has no significant effect on the amplitude of SOCE in HEK293-SK3$(CAG)_{24}$ (Fig. 1B). To test whether docetaxel increased the amplitude of SOCE of HEK293-SK3$(CAG)_{11}$ by increasing the activity of SK3 channel, we tested Ohmline (1-O-hexadecyl-2-O-methyl-sn-glycero-3-lactose) at 300 nmol/L, a lipid inhibitor of the activity of SK3 channel (9), on the amplitude of SOCE recorded in HEK cells. Figure 1C and D shows that Ohmline significantly reduced the amplitude of SOCE recorded in HEK293-SK3$(CAG)_{11}$ to a level similar to the amplitude of SOCE recorded in the absence of docetaxel (Fig. 1B). As expected, Ohmline has no significant effect on the amplitude of SOCE recorded in HEK293-SK3$(CAG)_{24}$ cells (Fig. 1D).

Patients

A total of 176 patients were enrolled in this study and their characteristics are summarized in Table 1. Among these patients, 3 received both types of taxanes over the course of their treatment. A change of chemotherapy was caused by hematologic or cutaneous toxicity. Median age was 54.2 years. Most of the patients (55.1%) received chemotherapies in an adjuvant setting, and docetaxel was the most frequently used taxane (77.3% of patients).

Neuropathy

No patients had any neuropathy prior to the chemotherapy initiation. Among 176 patients, during chemotherapy, 99 patients

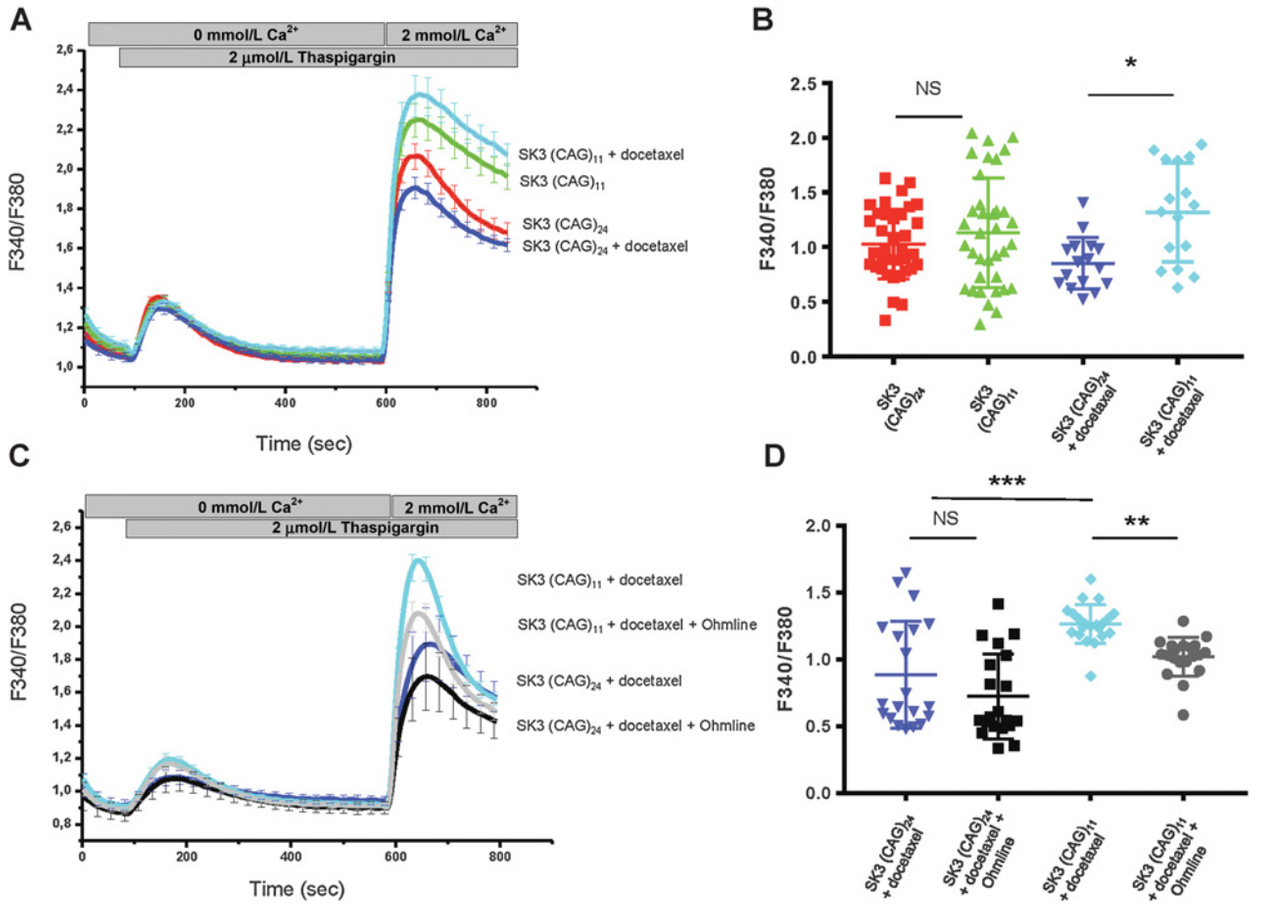

Figure 1.

Effect of docetaxel on SK3-dependent Ca^{2+} entry. Docetaxel was tested on Ca^{2+} influx in HEK293 cells that expressed SK3 $(CAG)_{11}$ or SK3 $(CAG)_{24}$ channels. **A,** Time-dependent fluorescence measurement of SOCE recorded in the absence or presence of docetaxel at 10 μmol/L, 20 minutes before calcium measurements. **B,** Histograms showing steady-state relative (to control) fluorescence to SOCE in the absence or presence of docetaxel at 10 μmol/L, 20 minutes before Ca^{2+} measurements. Data, means ± SEM. *, Significantly different from control at $P < 0.05$ ($N = 5$, $n = 36$ for conditions without docetaxel and $N = 4$, $n = 16$ for conditions with docetaxel, Kruskal–Wallis and *post hoc* tests). Ohmline, a SK3 channel blocker, was tested on docetaxel-treated Ca^{2+} influx in HEK293 cells that expressed SK3 $(CAG)_{11}$ or SK3 $(CAG)_{24}$ channels. **C,** Time-dependent fluorescence measurement of SOCE recorded in the absence or presence of Ohmline at 300 nmol/L, 20 minutes before calcium measurements. **D,** Histograms showing steady-state relative (to control) fluorescence to SOCE in the absence or presence of Ohmline at 300 nmol/L, 20 minutes before Ca^{2+} measurements. Data, means ± S.E.M. *, Significantly different from control at $P < 0.05$ ($N = 3$, $n = 20$ for SK3 $(CAG)_{24}$ and SK3 $(CAG)_{11}$ without Ohmline and $N = 3$, $n = 21$ for SK3 $(CAG)_{11}$ with Ohmline, Kruskal–Wallis and *post hoc* tests).

experienced paresthesia (56.2%) and when present, neuropathy was mainly of grade 1 (41.5%, 73 patients). According to the clinical evaluation, neuropathy was absent in 77 patients (G0, 43.7%), mild in 22 patients (G2, 12.5%), and severe in 4 patients (G3, 2.7%). No grade 4 were observed. Figure 2 shows that neuropathies appeared mostly during the first 6 months of treatment and that the likelihood for appearance of neuropathy did not differ between paclitaxel or docetaxel treatments. The mean cumulative dose received by patients when neurotoxicity appeared was 170.4 ± 349.1 mg/m^2 for paclitaxel and 130.7 ± 149.4 mg/m^2 for docetaxel.

Genetic findings and neuropathy

Fluorescent capillary electrophoresis provided excellent results with one (homozygous) or two peaks (heterozygous) for each patient, separated by a multiple of 3 units (Fig. 3A). A perfect concordance was obtained between the size of the amplicons

Table 1. Characteristics of the patients enrolled in the study

	***N* = 176**
Age	**54.2 ± 11.5 (30–88)**
BMI	**24.3 ± 4.6 (15.6–37.0)**
Chemotherapy	
Neoadjuvant	**48 (27.3%)**
Adjuvant	**97 (55.1%)**
Metastatic	**31 (17.6%)**
Taxane	
Paclitaxel	**43 (24.4%)**
Docetaxel	**136 (77.3%)**
Histologic type	
Ductal	**141 (80.1%)**
Lobular	**20 (11.4%)**
Other	**15 (8.5%)**

NOTE: Clinical and histologic features and type of chemotherapy. Other histologic types are mixed (ductal and lobular), mucinous, and papillary. Three patients received both taxane drugs.
Abbreviation: BMI, body mass index.

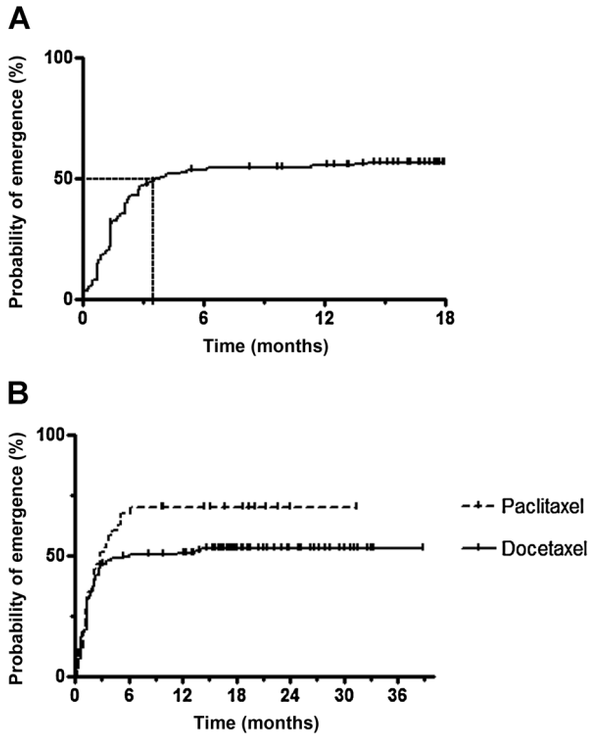

Figure 2.

Likelihood of neuropathy occurrence under taxane treatment. **A,** Probability of neuropathy development according to time after administration of docetaxel or paclitaxel. The occurrence of a peripheral neuropathy was recorded in 176 patients receiving a taxane-based chemotherapy for breast cancer. **B,** Probability of neuropathy development depending on the taxane used. No significant statistical difference between docetaxel and paclitaxel administration curves was observed.

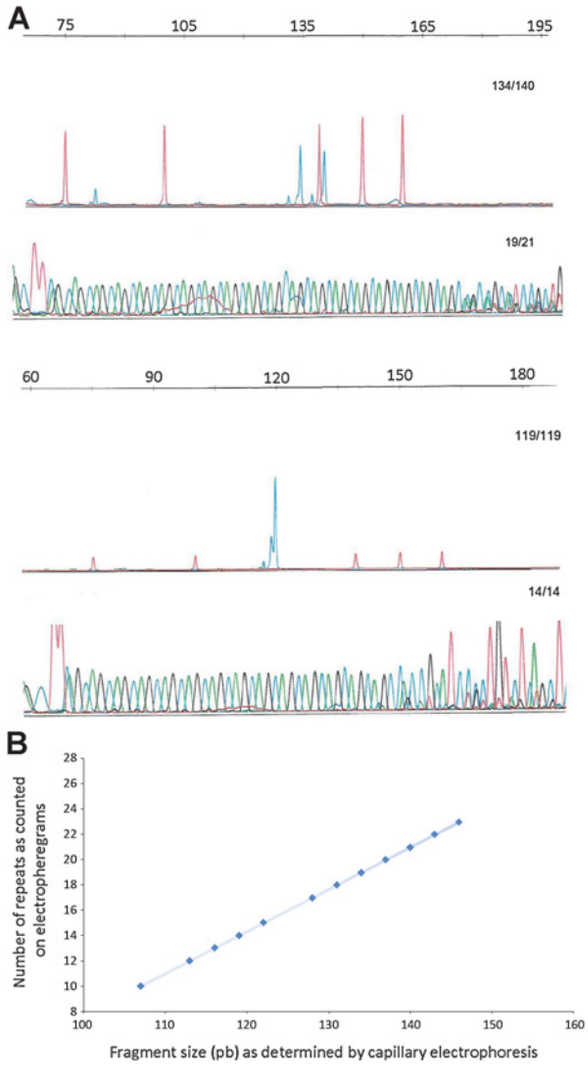

Figure 3.

A, Examples of genetic results obtained in 2 patients analyzed by fluorescent capillary electrophoresis and Sanger sequencing. Top, a heterozygous patient with fragments of 134 and 140 pb corresponding to 19 and 21 repeats, respectively. Bottom, a homozygous patient with fragments of 119 pb corresponding to 14 repeats. The number of repeats was easy to count on electropherograms, albeit more in homozygous than in heterozygous patients in whom CAG bases of the longer allele superimposed on non-CAG ones (see electropherogram of patient 19/21). For capillary electrophoresis results, blue peaks are those of the patient and red ones correspond to those of the ROX size standard. The blue fragments appeared two bases shorter than expected on the basis of the target DNA sequence, because of slight imprecision in size allocation from the ROX size standard (see patient with 14 repeats in whom the expected size was 121 pb and the estimated one 119). Comparison with Sanger sequencing allowed fixing the exact number of CAG repeats. **B,** Correspondence between capillary electrophoresis and Sanger sequencing determinations. The genotypes of 12 patients were determined using the two genotyping methods to check their comparability. This calibration curve was used to extrapolate the number of CAG repeats from any fragments sizes obtained by capillary electrophoresis.

assessed by fluorescent capillary electrophoresis and the number of CAG repeats obtained by electropherograms analysis in the 12 patients that were analyzed by the two methods (Fig. 3B).

In our cohort, 44 patients (25%) were homozygous for a type of CAG repeats (i.e., carried 2 alleles of the same length) and 135 (75%) were heterozygous. The number of CAG repeats ranged from 13 to 21, with 19 CAG repeats being the most frequent (Fig. 4). The sum of both CAG allele repeats ranged from 26 to 41, with 36–38 being the most frequent and a median around 37 (Fig. 5A). We found a significant difference in neuropathy between patients carrying long forms (sum of CAG repeats $\geq$ 37) versus short (sum of CAG repeats <37; Fig. 5B). Indeed, a shorter CAG sum was associated with an increased probability of neuropathy ($P = 0.036$) with 65.9% of patients with short CAG sums experiencing neuropathies as compared with 47.3% of those with long CAG sums [risk ratio (RR) = 1,51; 1.02; 2.3].

To confirm that patients carrying alleles containing short CAG repeats experienced increased neuropathy incidence than patients carrying long CAG repeats, we chose to consider only the homozygous population, that is, patients with the same CAG repeat number for both alleles. Figure 5C shows that CAG repeat numbers in homozygous patients ranged from 13 to 20, with 18–19 CAG repeat being the most frequent. The median of the CAG repeat is 19 corresponding to the median of the CAG repeat sum of 38. As expected, short CAG alleles (repeats < 19 CAG) were highly associated with neuropathy incidence as compared with long CAG ones (repeats $\geq$ 19 CAG; $P = 0.016$; Fig. 5D). A total of 84.6% of patients carrying two short alleles experienced

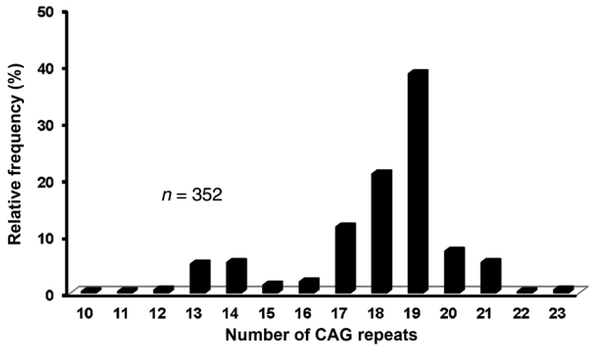

Figure 4.
Distribution of *KCNN3*-CAG repeat numbers for each allele in four cohorts of patients receiving taxane chemotherapy for breast cancer. The distribution of *KCNN3*-GAG repeats numbers was recorded in 176 patients receiving a taxane-based chemotherapy for breast cancer. Although the number of CAG repeats ranged from 10 to 23, 19 CAG repeats was the most frequent allele.

neuropathy compared with 48.4% of patients with two long alleles (RR = 2.5; 1.2; 8.1).

Discussion

We report that patients receiving taxanes during chemotherapy for breast cancer have a different likelihood of developing a peripheral neuropathy according to the CAG polymorphism of *KCNN3* encoding the SK3 channel. Patients with short *KCNN3* CAG alleles have a significantly higher incidence of taxane neuropathy as compared with patients carrying longer alleles. Consequently, a CAG polymorphism of the SK3 channel is associated with taxane-induced peripheral neurotoxicity.

Taxane-induced peripheral neuropathy is a limiting factor for the use of taxanes such as paclitaxel and docetaxel because they impact patient's quality-of-life. Indeed, some patients experience invalidating neuropathy, which is sometimes irreversible, or may last years after the end of chemotherapy. The pathophysiology of taxane-induced peripheral neuropathy is not well understood and diverse mechanisms have been proposed.

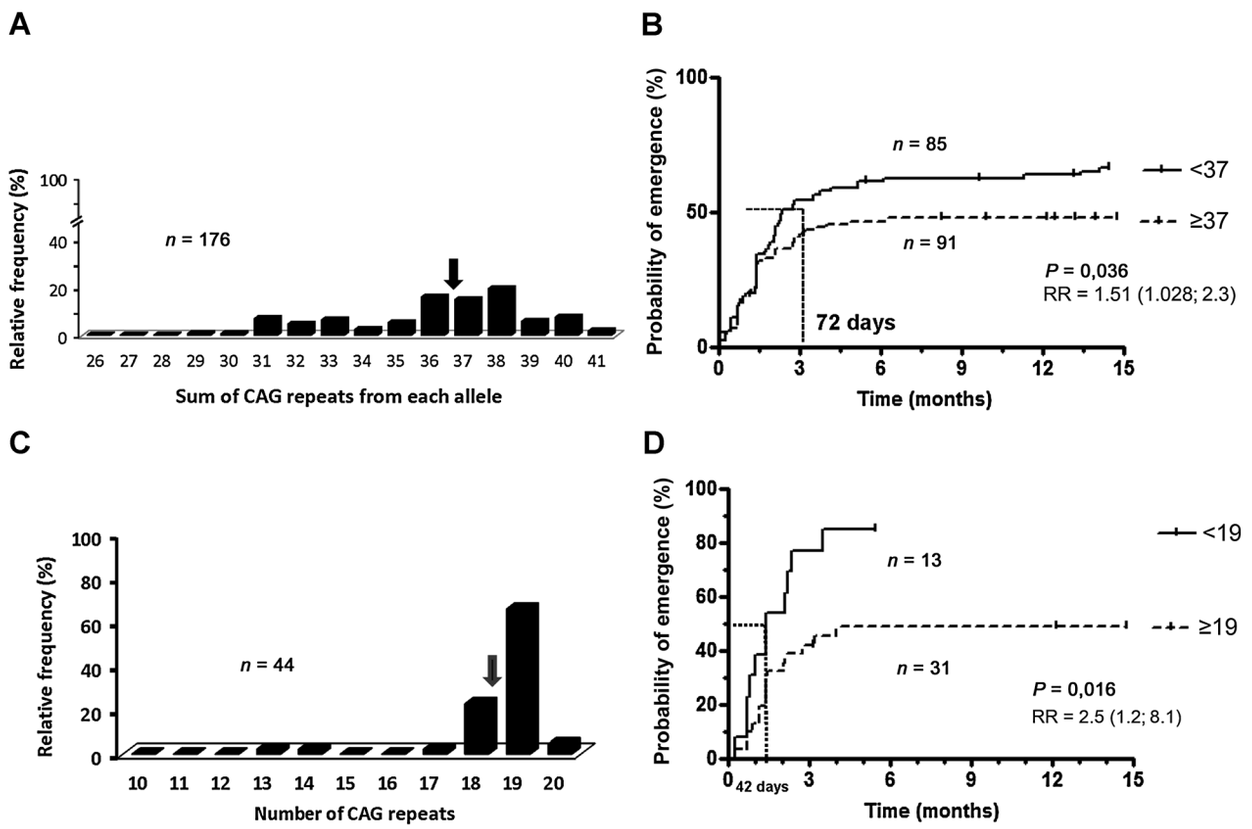

Figure 5.
A and **B,** Distribution of the sum of KCNN3-CAG repeats for each patient in the cohort and the associated probability of experiencing neuropathy. **A,** Distribution of the sum of CAG repeats from each allele recorded for the 176 patients receiving a taxane-based chemotherapy for breast cancer. Although the number of CAG repeats ranged from 26 to 41, the median was 37. **B,** Probability of neuropathy development according to the sum of CAG repeats. A significant difference between patients carrying long and short forms of CAG repeats was observed. **C** and **D,** Distribution of CAG repeats in the homozygous population and the associated probability of experiencing neuropathy. **C,** Distribution of CAG repeats for each patient. Although the number of CAG repeats ranged from 13 to 20, the median was 19. **D,** Probability of neuropathy development according to CAG repeat numbers. A significant difference between patients carrying long and short forms of CAG repeats was observed.

Interestingly, our results show that in the presence of docetaxel, Ca^{2+} entry is significantly higher in cells expressing short CAG alleles of SK3 channel than the long forms. Because it was demonstrated that long CAG alleles in the *KCNN3* gene lead to a reduction of SK3 channel activity (15), we propose that the increased Ca^{2+} entry induced by docetaxel is linked to the activity of SK3 channel. Interestingly, Ohmline reduced the amplitude of SOCE that is increased by docetaxel in cells expressing short CAG alleles in the *KCNN3* gene to a same level as the amplitude of SOCE recorded in the absence of docetaxel. Because we found that Ohmline is acting in active SK3 channel, (9) this strongly suggests that the increased Ca^{2+} entry induced by docetaxel is linked to the activity of SK3 channel. This finding is in agreement with the observation that docetaxel and paclitaxel can both activate voltage-independent Ca^{2+} channel such as transient receptor potential channels TRPV4 (5), TRPA1 (17), and the voltage-gated Ca^{2+} channel (CaV3.2; ref. 6). The increased Ca^{2+} entry induced by taxanes may in turn activate Ca^{2+}-sensitive proteases, leading to the degeneration of peripheral and central branches of dorsal root ganglia axons, as observed previously (3, 4). We have already reported that the SK3 channel, which is expressed in dorsal root ganglion neurons (13), promotes Ca^{2+} entry and the activity of Ca^{2+}-activated proteases calpains (9). Our hypothesis is that docetaxel increases the activity of SK3 and/or voltage-independent Ca^{2+} channel in dorsal root ganglion neurons. This activity could increase cytosolic Ca^{2+} concentrations that in turn activate SK3 channels. As a result, activation of the SK3 channel may therefore be enhanced in cells expressing short CAG repeat alleles compared with the long forms. Therefore, hyperpolarization of the plasma membrane due to short SK3 channel forms increases the Ca^{2+} driving force and consequently favors Ca^{2+} entry. A positive feedback loop between Ca^{2+} and SK3 channels may ensue, and it may be more efficient in cells expressing short SK3 CAG channels than long forms.

The electrophysiologic properties of SK3 channels differ between short and long forms of the SK3 channel. A short CAG repeat number in the *KCNN3* gene leads to increased SK3 channel activity (15). However, it is not known how the N-terminus domain of the SK3 channel can interfere with the permeation properties of the channel. In heterozygous population, we do not know the phenotypic effect of different *KCNN3* CAG repeat numbers on the SK3 channel activity. Because this complex is formed by the association of four homo or hetero alpha subunits, the most likely association is a heterotetramerization between different CAG repeats lengths. If such an association may be observed in patients, it was not the case in the HEK293 cell lines that are homozygous.

The number of CAG repeats in *KCNN3* we have measured in patients is in agreement with their prevalence described among Caucasian populations (8, 15, 18, 19). The increased SK3 channel activity observed for short *KCNN3* alleles in patients with breast cancer is associated with a significantly increased incidence of taxane neuropathy compared with patient carrying long alleles. This association was already observed in patients receiving oxaliplatin for colorectal cancer (8). To our knowledge, our study is the first one that combines genetics and *in vitro* data to demonstrate that *SK3* gene polymorphism is associated with taxane neurotoxicity. In the current study, we chose to include all patients with taxane treatment because we did not expect major differences between docetaxel and paclitaxel in term of neuropathy and genetics findings because the two drugs are members of the same pharmacologic family. We found no significant difference in neuropathy incidence between the two drugs, and this finding is in agreement with results showing that patients receiving paclitaxel and docetaxel experienced similar symptoms of taxane-induced peripheral neuropathy (20).

In our study, we found that the CAG polymorphism of *KCNN3* is associated with taxane-induced peripheral neurotoxicity. Determination of *KCNN3* polymorphism in patients prior to taxane administration may therefore help identify those at risk of developing taxane-induced peripheral neuropathy. For these patients, another type of chemotherapy or schedule of administration could be proposed. Understanding the mechanism of chemotherapy-induced peripheral neuropathy is also important to develop new drugs capable of preventing or treating taxane-induced peripheral neuropathy. Confirmation of the role of the SK3 channel in taxane-induced peripheral neuropathy could lead to the use of inhibitors of the SK3 channel in patients carrying a short polymorphism to prevent or treat these neuropathies. We have already developed synthetic alkyl-ether-lipids, such as Ohmline, that target cell membranes and are specific and nontoxic inhibitors of the SK3 channel (21).

In conclusion, even if there is a need to confirm these observations, this study shows for the first time the presence of a genetic predisposition to develop taxane-induced neurotoxicity, and we identified a mechanism of action that involves the effect of taxanes on SK3-dependent Ca^{2+} regulation. As a next step, it will be important to determine whether the expression of long and short SK3 channels affects dorsal root ganglion neurons and if so, to test the effect of specific SK3 blockers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C. Rua, M. Guéguinou, E. Viel, A. Chantome, P. Bougnoux, C. Barin-Le Guellec, C. Vandier
Development of methodology: C. Rua, E. Viel, C. Barin-Le Guellec, C. Vandier
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Rua, M. Guéguinou, I. Soubai, E. Viel, C. Barbe, C. Barin-Le Guellec
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Rua, M. Guéguinou, A. Chantome, P. Bougnoux, C. Barin-Le Guellec
Writing, review, and/or revision of the manuscript: C. Rua, M. Guéguinou, E. Viel, M. Potier-Cartereau, A. Chantome, P. Bougnoux, C. Barin-Le Guellec, C. Vandier
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Rua
Study supervision: C. Rua, C. Vandier

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References

1. Cavaletti G, Marmiroli P. Chemotherapy-induced peripheral neurotoxicity. Nat Rev Neurol 2010;6:657–66.
2. Kuroi K, Shimozuma K. Neurotoxicity of taxanes: symptoms and quality of life assessment. Breast Cancer 2004;11:92–9.
3. Tasnim A, Rammelkamp Z, Slusher AB, Wozniak K, Slusher BS, Farah MH. Paclitaxel causes degeneration of both central and peripheral axon branches of dorsal root ganglia in mice. BMC Neurosci 2016;17:47.
4. Wang MS, Davis AA, Culver DG, Wang Q, Powers JC, Glass JD. Calpain inhibition protects against Taxol-induced sensory neuropathy. Brain 2004;127:671–9.
5. Alessandri-Haber N, Dina OA, Yeh JJ, Parada CA, Reichling DB, Levine JD. Transient receptor potential vanilloid 4 is essential in chemotherapy-induced neuropathic pain in the rat. J Neurosci 2004;24:4444–52.
6. Okubo K, Takahashi T, Sekiguchi F, Kanaoka D, Matsunami M, Ohkubo T, et al. Inhibition of T-type calcium channels and hydrogen sulfide-forming enzyme reverses paclitaxel-evoked neuropathic hyperalgesia in rats. Neuroscience 2011;188:148–56.
7. Sun T, Song ZG, Jiang DQ, Nie HG, Han DY. Docetaxel modulates the delayed rectifier potassium current (IK) and ATP-sensitive potassium current (IKATP) in human breast cancer cells. J Membr Biol 2015;248: 197–204.
8. Basso M, Modoni A, Spada D, Cassano A, Schinzari G, Lo Monaco M, et al. Polymorphism of CAG motif of SK3 gene is associated with acute oxaliplatin neurotoxicity. Cancer Chemother Pharmacol 2011;67:1179–87.
9. Chantome A, Potier-Cartereau M, Clarysse L, Fromont G, Marionneau-Lambot S, Gueguinou M, et al. Pivotal role of the lipid Raft SK3-Orai1 complex in human cancer cell migration and bone metastases. Cancer Res 2013;73:4852–61.
10. Gueguinou M, Harnois T, Crottes D, Uguen A, Deliot N, Gambade A, et al. SK3/TRPC1/Orai1 complex regulates SOCE-dependent colon cancer cell migration: a novel opportunity to modulate anti-EGFR mAb action by the alkyl-lipid Ohmline. Oncotarget 2016;7:36168–84.
11. Monaghan AS, Benton DC, Bahia PK, Hosseini R, Shah YA, Haylett DG, et al. The SK3 subunit of small conductance Ca2+-activated K+ channels interacts with both SK1 and SK2 subunits in a heterologous expression system. J Biol Chem 2004;279:1003–9.
12. Chen MX, Gorman SA, Benson B, Singh K, Hieble JP, Michel MC, et al. Small and intermediate conductance Ca(2+)-activated K+ channels confer distinctive patterns of distribution in human tissues and differential cellular localisation in the colon and corpus cavernosum. Naunyn Schmiedebergs Arch Pharmacol 2004;369:602–15.
13. Bahia PK, Suzuki R, Benton DC, Jowett AJ, Chen MX, Trezise DJ, et al. A functional role for small-conductance calcium-activated potassium channels in sensory pathways including nociceptive processes. J Neurosci 2005;25:3489–98.
14. Stocker M. Ca(2+)-activated K+ channels: molecular determinants and function of the SK family. Nat Rev Neurosci 2004;5:758–70.
15. Grube S, Gerchen MF, Adamcio B, Pardo LA, Martin S, Malzahn D, et al. A CAG repeat polymorphism of KCNN3 predicts SK3 channel function and cognitive performance in schizophrenia. EMBO Mol Med 2011;3: 309–19.
16. Hosseini R, Benton DC, Dunn PM, Jenkinson DH, Moss GW. SK3 is an important component of K(+) channels mediating the afterhyperpolarization in cultured rat SCG neurones. J Physiol 2001;535(Pt 2):323–34.
17. Huang K, Bian D, Jiang B, Zhai Q, Gao N, Wang R. TRPA1 contributed to the neuropathic pain induced by docetaxel treatment. Cell Biochem Funct 2017;35:141–3.
18. Chandy KG, Fantino E, Wittekindt O, Kalman K, Tong LL, Ho TH, et al. Isolation of a novel potassium channel gene hSKCa3 containing a polymorphic CAG repeat: a candidate for schizophrenia and bipolar disorder? Mol Psychiatry 1998;3:32–7.
19. Wittekindt O, Jauch A, Burgert E, Scharer L, Holtgreve-Grez H, Yvert G, et al. The human small conductance calcium-regulated potassium channel gene (hSKCa3) contains two CAG repeats in exon 1, is on chromosome 1q21.3, and shows a possible association with schizophrenia. Neurogenetics 1998;1:259–65.
20. Tofthagen C, McAllister RD, Visovsky C. Peripheral neuropathy caused by paclitaxel and docetaxel: an evaluation and comparison of symptoms. J Adv Pract Oncol 2013;4:204–15.
21. Jaffres PA, Gajate C, Bouchet AM, Couthon-Gourves H, Chantome A, Potier-Cartereau M, et al. Alkyl ether lipids, ion channels and lipid raft reorganization in cancer therapy. Pharmacol Ther 2016;165:114–31.