RESEARCH ARTICLE

Association of Voltage-Gated Sodium Channel Genetic Polymorphisms with Oxaliplatin-Induced Chronic Peripheral Neuropathy in South Indian Cancer Patients

Sreenivasulu Palugulla1, Dimpal N Thakkar1, Smita Kaylor2, Sunil K Narayan3, Steven Aibor Dkhar4*

Abstract

Oxaliplatin is a platinum drug active against digestive tract cancers. Among its side effects, peripheral neuropathy is one of the dose-limiting toxicities. This affects around 50 to 70% of patients but the pathophysiology of development of oxaliplatin-induced peripheral neuropathy (OXAIPN) remains unclear. Sodium channels (SCNAs) play major role in neuronal electrical signaling processes and mutations in SCNAs lead to various neuronal diseases involving the central and peripheral nervous systems. In this study, we evaluated whether SCNA genetic variants might be associated with risk of chronic OXAIPN in patients with digestive tract cancers treated with oxaliplatin. **Methodology:** Blood samples from 228 digestive tract cancer patients who had received oxaliplatin in adjuvant and neoadjuvant or metastatic settings were obtained and genomic DNA was extracted by phenol-chloroform extraction. Genotyping was performed with the real-time polymerase chain reaction (RT-PCR) using validated real-time TaqMan single nucleotide polymorphism (SNP) genotyping assays. Neuropathy was evaluated and graded according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 4.03. **Results:** We found that the rs6746030 polymorphic variant of SCN9A was significantly associated with a higher incidence of chronic OXAIPN (GA+AA vs GG: OR=1.8, 95% CI=1.04-3.4, P=0.04; dominant model) while the rs6754031 variant was linked with a lower incidence (OR=0.45, 95% CI=0.22-0.77, P=0.005; dominant model). The SCN 10A polymorphic variant was associated with severity of chronic OXAIPN (P=0.006, OR=2.0, 95% CI=1.2 - 3.3). **Conclusion:** The results of the present prospective study provide evidence in support of a causal relationship between chronic OXAIPN and voltage gated sodium channel polymorphisms. However, further studies from independent groups are required to validate these results.

**Keywords:** Oxaliplatin- single nucleotide polymorphisms- peripheral neuropathy- sodium channels- cancer

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Introduction

Oxaliplatin (OXA) is a novel platinum chemotherapeutic drug widely used in the treatment of various solid tumours mainly in digestive tract cancer (Hill et al., 2012). It induces one of the dose-limiting toxicities called peripheral neuropathy during or after chemotherapy. OXA-induced peripheral neuropathy (OXAIPN) represents as acute and chronic neuropathy. Acute neuropathy is a transient syndrome that is characterized by cold triggered paraesthesia and dysesthesias along with jaw spasms and cramps. Acute OXAIPN is usually reversible and resolves within the same cycle or later. It requires prolongation of OXA infusion when it is severe (Argyriou et al., 2008; Lehky et al., 2004).

Chronic type is purely sensory OXAIPN with a stocking and glove distribution. It develops gradually upon infusion of OXA and last for a long time or sometimes may persist even after completion of the treatment due to the accumulation of OXA in dorsal root ganglion of neurons (Argyriou et al., 2008). It is characterised by distal paraesthesia and dysesthesias and occurs at a threshold of 600-700 mg/m2 of the cumulative dose of OXA and affects between 50% and 70% of the patients (André et al., 2004; Argyriou et al., 2010). It became clinically important as many patients exhibit significant functional difficulties with daily activities that affect their quality of life (Cavaletti et al., 2013).

The exact molecular basis for underlying oxaliplatin-induced polyneuropathy (OXAIPN) remains unclear (Zedan et al., 2014). Though clinical predictors for OXAIPN have been suggested, to date no reliable pharmacogenetics or molecular biomarkers have been identified with which to identify the patients who are at high risk of developing OXAIPN. To date, several single nucleotide polymorphisms (SNPs) in various genes are

1Department of Pharmacology, 2Department of Medical Oncology, 3Department of Neurology, 4Department of Clinical Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India.

*For Correspondence: steven.jipmer16@gmail.com
identified in the context of the toxicity and efficacy of OXA. These include genes encoding enzymes associated with detoxification or those linked with DNA repair pathways, membrane efflux proteins or voltage gated sodium channels (Argyriou et al., 2009, 2013a; Inada et al., 2010). However, these genes have not been validated in prospective studies.

The voltage gated sodium channels (SCNAs) play major role in electrical signaling processes in neurons and other excitable cells. To date nine sodium channel subunits have been identified (SCN1A-SCN5A, SCN8A-SCN11A). They are well distributed in dorsal root ganglion neurons and their axons of both central and peripheral nervous systems.

It is now well recognized that mutations in these SCNAs have been associated with various diseases like epilepsy, ataxia, myotonia, pain and paroxysmal extreme pain disorders in both central and peripheral nervous systems (Hoeijmakers et al., 2015; Waszkielewicz et al., 2013).

Genes like SCN4A, SCN5A, SCN9A, SCN10A, encode Nav1.4, Nav 1.5, Nav 1.7, Nav 1.8 isoforms respectively. During the last few years, the role of these four gene mutations were extensively addressed in painful neuropathy (Palmio et al., 2017). To date, there is limited data concerning the association of development of OXAIPN with voltage gated SCN polymorphisms. Taking into account these findings, in the present study, we aimed to explore the association between SCN4A (rs2302237), SCN5A (rs1720524), SCN9A (rs6746030, rs6754031), SCN10A (rs12632942) SNPs and development of severe chronic OXAIPN in south Indian GIT cancer patients who are treated with oxaliplatin based chemotherapy.

Materials and Methods

Study design and patient selection

This is a prospective cohort study. This study was carried out in 228 histologically confirmed gastrointestinal tract (GIT) cancer patients of either gender who were scheduled to receive oxaliplatin-based chemotherapeutic treatment at Regional Cancer Centre (RCC) in collaboration with the departments of pharmacology, neurology and Medical Oncology at JIPMER (Jawaharlal Institute of Postgraduate Medical Education and Research) India, from November 2014 to December 2016. All patients were followed up from the start of the oxaliplatin based chemotherapy until the occurrence of chronic OXAIPN or death or till completion of the treatment. Patients who were less than 18 years of age, patients with eastern cooperative oncology group (ECOG) performance status greater than 2, patients with abnormal hepatic and renal functions, patients with abnormal electrophysiological profile, pregnant or lactating women and patients with neuropathy or receiving other drugs which cause neuropathy were excluded from the study. The study was approved by Institute ethics committee (Reg.No: ECR/342/Inst/PY/2013), JIPMER and was conducted with the ethical standards of the Declaration of Helsinki. Written informed consent was taken from all the participants before including them into the study.

Treatment protocol and end points in the study

The allotment of GIT cancer patients to the treatment of OXA based chemotherapy was made according to treating medical oncologist’s discretion. For CAPOX (capecitabine and oxaliplatin) and EOX (Epirubicin, Capecitabine and oxaliplatin) of OXA based chemotherapy regimens, OXA was administered at 130 mg/m², three weekly, for eight cycles. For FOLFOX (leucovorin, 5-fluorouracil and oxaliplatin) and GEMOX (Gemcitabine and oxaliplatin) of OXA based chemotherapy regimens, OXA was infused intravenously for 2 hours at 85 and 100mg/m², two weekly, for twelve cycles. Development of grade 4 chronic OXAIPN was the end point of the present study.

Assessment of oxaliplatin induced peripheral neuropathy in the study cohort

Patients who were included in the study underwent clinical evaluation at baseline (visit1) and during the course of treatment (at every cycle) to detect the OXAIPN. The patients who developed OXAIPN were considered as cases and the patients who did not develop OXAIPN were considered as controls. Chronic OXAIPN was graded according to the Common Terminology Criteria for Adverse Events, version 4.03.

DNA extraction and genotyping of selected SNPs in the study

Approximately five millilitre of peripheral venous blood was collected from each study patients in tubes containing 100 µL of 10% ethylene diamine tetra acetic acid (EDTA) and centrifuged at 2500 RPM for 5 minutes. The plasma was discarded and the pellets containing lymphocytes in the form of buffy coat along with red blood cells were stored at -80 °C in the laboratory deep freezer until DNA extraction. The genomic DNA for genotyping was extracted from the peripheral lymphocytes by phenol–chloroform extraction method (Blin and Stafford, 1976) and was quantified by using multi-analyzer (TECAN Infinite M200, Switzerland).

The genotyping was carried out by Real-Time PCR (ABI Prism 7300, foster city, CA, USA) using validated Real-Time TaqMan single nucleotide polymorphism (SNP) genotyping assays (Applied Biosystems, Foster City, CA, USA) according to manufacturer instructions. The version 1.4 of 7300 sequence detection software was used for allelic discrimination. All samples were analysed in duplicates along with negative controls to ensure the authenticity of the results and the results were found to be 100% consistent. The details of various SNPs of SCNAs screened in this study are given in Table 1.

Statistical analyses

We used descriptive statistics for the analysis of all variables (mean, standard deviation, percentage distribution). Clinical variables among patients who developed and who did not develop chronic OXAIPN were compared using Student’s t-test or Mann-Whitney U test (age and cumulative dose of oxaliplatin) or Chi square test (gender, presence of diabetes mellitus, tumour type, type of treatment and tumour stage). Fisher’s exact test was employed to determine the influence of different genotypes
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Allelic frequency and genotype distributions of all the SNPs of SCNAs are given in Table 4. Coming to association between SNPs genotyping and of various SCN polymorphisms with the incidence and severity of chronic OXAIPN. Allele frequency and genotype distributions for each SNP were also tested with chi square test for Hardy–Weinberg equilibrium (HWE) probability. Graph Pad Instat version 3.0 (Graph Pad Software Inc., San Diego, CA, USA) was used for all the statistical analyses. Two sided p value <0.05 was considered statistically significant.

**Results**

**Characteristics of the study patients**

A total of 228 GIT cancer patients were recruited in the study in a 26 month period between November 2014 and December 2016. Among 228 patients, there were 86 females and 142 males in the study with a median age of 53 (range: 19-75) years. Out of 228 cancer patients, 108 patients were from colon and rectal cancers, 111 patients were from gastric cancers and 9 patients were from pancreatic and biliary cancers, respectively. All the patients received a median dose of oxaliplatin at 780 mg/m$^2$. The detailed demographic and clinical characteristics are given in Table 2.

**Incidence and severity of chronic OXAIPN**

Chronic OXAIPN was manifested in 130 (57.0%) of 228 cancer patients. The distribution of incidence and severity of chronic OXAIPN was graded at the final follow up i.e. at the end of chemotherapy. Grade 1 was found in 73 (56.1%) patients, grade 2 in 55(42.3%) patients and grade 3 in 2 (1.6%) patients respectively. The median time for the onset of chronic OXAIPN was 105 (42–168) days. In the study cohort the occurrence of chronic OXAIPN was related to the cumulative dose of OXA and patients receiving ≥ 780mg/m$^2$ of OXA dose had a higher incidence rate of chronic OXAIPN (p=0.0001). Other demographic and clinical factors such as age, gender, performance status, comorbidity, site of cancer and type of chemotherapy regimen, were not associated with the occurrence of chronic OXAIPN (Table 3).

The genotype distribution, allele frequencies of SCNAs SNPs and their association with chronic OXAIPN in the study cohort

In the present study, genotypes of 5 SNPs of SCNAs correlated with the incidence and severity of chronic OXAIPN. Association between genotype and chronic OXAIPN were tested in different genetic models (Lewis, 2002) by using Fisher’s exact test. With regarding genotyping distribution, all the 5 SNPs of SCNAs were within Hardy–Weinberg equilibrium (HWE) probability.

| Gene   | SNP type | SNP ID/ rs ID | Allele change | Gene location | Assay ID       |
|--------|----------|---------------|---------------|---------------|---------------|
| SCN4A  | Intron   | 2302237       | C/T           | Chr.17:63971347 | C__15757352_10 |
| SCN5A  | Intron   | 11720524      | C/G           | Chr.2:240878099 | C__30666704_10 |
| SCN9A  | Missense | 6746030       | G/A           | Chr.2:166242648 | C__29330435_10 |
| SCN9A  | Intron   | 6754031       | T/G           | Chr.2:166298928 | C__29108389_10 |
| SCN10A | Missense | 12632942      | A/G           | Chr.3:38723507  | C__31683397_10 |

| Variable | No. of patients (%) |
|----------|----------------------|
| Age      | Median (range), years | 53 (19-75) |
| Gender   | Male                  | 142 (62.3) |
|          | Female                | 86 (37.7)  |
| ECOG-PS  | 0                     | 22 (9.6)   |
|          | 1                     | 175 (76.8) |
|          | 2                     | 31 (13.6)  |
| Height, (CM) | 157.42±8.45        |
| Weight, (Kg)  | 49.37±9.77          |
| BSA, (m$^2$) | 1.51±0.91           |
| Comorbidity | Nil                   | 192        |
|           | Diabetes mellitus    | 19         |
|           | Hypertension         | 16         |
|           | Bronchial asthma     | 2          |
|           | Hypothyroid          | 2          |
| Site of cancer | Stomach              | 111 (48.7) |
|           | Colorectal           | 108 (47.4) |
|           | Gall bladder and pancreas | 9 (3.9)   |
| Stage of cancer: n,% | Metastatic           | 106 (46.5) |
|          | Non-metastatic       | 122 (53.5) |
| Type of chemotherapy | Adjuvant              | 85 (37.3)  |
|           | Neoadjuvant          | 30 (13.1)  |
|           | Palliative           | 113 (49.6) |
| Type of regimen | CAPOX                 | 123 (53.9) |
|           | EOX                  | 76 (33.3)  |
|           | FOLFOX               | 20 (8.8)   |
|           | GEMOX                | 9 (4.0)    |
| Median dose (range) n, % | 780 mg/m$^2$ | (260-1040mg/m$^2$) |

ECOG-PS, Eastern Cooperative Oncology Group Performance Status; BSA, Body Surface Area
chronic OXAIPN, polymorphic variant of rs6746030 of SCN9A was significantly associated with higher incidence of chronic OXAIPN in two genetic models (AG vs GG: OR=2.16, 95%CI=1.14-4.06, P=0.02 and GA+AA vs GG: OR=1.8, 95%CI=1.04-3.4, P=0.04) but when we tested for severity of chronic OXAIPN the same SNP did not show any statistical significance.

For rs6754031 polymorphism of SCN9A, we found that patients harbouring G polymorphic variant had lower risk of both the incidence and severity of chronic OXAIPN when compared to wild type TT genotype in dominant (TG+GG vs TT) and over dominant (TG vs TT+GG) models (dominant model: incidence, OR=0.45, 95%CI=0.22-0.77, P=0.005; severity, OR=0.43, 95%CI=0.22-0.77, P=0.005).

Table 3. Association of Chronic OXAIPN with Baseline and Clinical Variables

| variable                              | Total no. of patients; N=228(%) | Chronic OXAIPN | P value |
|---------------------------------------|---------------------------------|----------------|---------|
| Age                                   |                                 |                |         |
| Median 53                             |                                 |                |         |
| ≤53                                   | 122 (53.5)                      | 45 (45.9)      | 77 (59.2) | 0.06 |
| >53                                   | 106 (46.5)                      | 53 (54.1)      | 53 (40.8) |       |
| Gender                                |                                 |                |         |
| Male                                  | 142 (62.3)                      | 61 (62.2)      | 81 (62.3) | 0.9  |
| Female                                | 86 (37.7)                       | 37 (37.8)      | 49 (37.7) |       |
| ECOG-PS                               |                                 |                |         |
| 0                                     | 22 (9.6)                        | 12 (12.2)      | 10 (7.7)  | 0.75 |
| 1                                     | 175 (76.8)                      | 72 (73.5)      | 103 (79.2)|       |
| 2                                     | 31 (13.6)                       | 14 (14.3)      | 17 (13.1) |       |
| Site of cancer                        |                                 |                |         |
| Stomach                               | 111 (48.7)                      | 44 (44.9)      | 67 (51.5) | 0.2  |
| Colorectal                            | 108 (47.4)                      | 48 (49.0)      | 60 (46.2) |       |
| Gall bladder/pancreas                 | 9 (4.0)                         | 6 (6.1)        | 3 (2.3)   |       |
| Type of chemotherapy                  |                                 |                |         |
| Adjuvant                              | 85 (37.3)                       | 37 (37.8)      | 48 (36.9) | 0.75 |
| Neoadjuvant                           | 30 (13.2)                       | 11 (11.2)      | 19 (14.6) |       |
| Palliative                            | 113 (49.5)                      | 50 (51.0)      | 63 (48.5) |       |
| Type of regimen                       |                                 |                |         |
| CAPOX                                 | 123 (53.9)                      | 46 (46.9)      | 77 (59.2) | 0.1  |
| EOX                                   | 76 (33.3)                       | 34 (34.7)      | 42 (32.3) |       |
| FOLFOX                                | 20 (8.8)                        | 12 (12.3)      | 8 (6.2)   |       |
| GEMOX                                 | 9 (4.0)                         | 6 (6.1)        | 3 (2.3)   |       |
| Median dose                           |                                 |                |         |
| 780mg/m²                              |                                 |                |         |
| <780                                  | 90 (39.5)                       | 53 (54.1)      | 37 (28.5) | 0.0001|
| ≥780                                  | 138 (60.5)                      | 45 (45.9)      | 93 (71.5) |       |

* p value < 0.05 is statistically significant; ECOG-PS, Eastern Cooperative Oncology Group Performance Status; BSA: Body Surface Area

Table 4. Allele and Genotype Frequencies of Selected SNPs in the Study Population

| Gene and SNP (rsID) | Wild type homogenous genotype n(%) | Mutant heterogeneous genotype n(%) | Mutant homogenous genotype n(%) | Dominant allele n(%) | Recessive allele n(%) |
|---------------------|------------------------------------|-----------------------------------|---------------------------------|----------------------|----------------------|
| SCN 4A              | CC                                 | CT                                | TT                              | C                    | T                    |
| rs2302237           | 176 (77.2)                         | 46 (20.2)                         | 6 (2.6)                         | 398 (87.3)           | 58 (12.7)            |
| SCN5A               | CC                                 | CG                                | GG                              | C                    | G                    |
| rs11720524          | 165 (72.4)                         | 52 (22.8)                         | 11 (4.8)                        | 382 (83.8)           | 74 (16.2)            |
| SCN9A               | GG                                 | GA                                | AA                              | GG                   | A                    |
| rs6746030           | 160 (70.2)                         | 61 (26.8)                         | 7 (3.0)                         | 381 (83.6)           | 75 (16.4)            |
| SCN9A               | TT                                 | TG                                | GG                              | T                    | G                    |
| rs6754031           | 109 (47.8)                         | 89 (39.0)                         | 30 (13.2)                       | 307 (67.3)           | 149 (32.7)           |
| SCN10A              | AA                                 | AG                                | GG                              | A                    | G                    |
| rs12632942          | 144 (63.2)                         | 78 (34.2)                         | 6 (2.6)                         | 366 (80.2)           | 90 (19.8)            |
Table 5: Association between Selected SNPs of SCNAs and Chronic OXAIPN (n=228)

| Gene and SNP | Severity of OXAIPN | p value | Incidence of OXAIPN | p value |
|--------------|--------------------|---------|---------------------|---------|
| SCN4A– C˃T rs2302237 | Grade 0 | 98 (77.2%) | Grade 1 | 74 (75.5%) | Grade 2 | 60 (82.2%) | Grade 3 | 41 (74.5%) | OR: 1.15 (0.60-2.22) | 0.78 |
| SCN5A-C ˃G rs11720524 | Grade 0 | 165 (72.4%) | Grade 1 | 76 (77.6%) | Grade 2 | 46 (63.0%) | Grade 3 | 44 (80.0%) | OR: 0.70 (0.37-1.34) | 0.37 |
| SCN9A- G˃A rs6746030 | Grade 0 | 160 (70.2%) | Grade 1 | 76 (77.6%) | Grade 2 | 47 (64.4%) | Grade 3 | 35 (63.7%) | OR: 0.46 (0.24-0.87) | 0.02* |
| SCN9A- T˃G rs6754031 | Grade 0 | 109 (47.8%) | Grade 1 | 36 (36.7%) | Grade 2 | 37 (50.7%) | Grade 3 | 35 (63.7%) | OR: 2.19 (1.21-3.86) | 0.01* |

*Significant p value.
CI = 0.23-0.80, p = 0.01; over dominant model: incidence, OR=0.5, 95% CI = 0.32 - 0.9, p = 0.04, severity; OR=0.36, 95% CI = 0.18-0.73, p = 0.006. SCN10A (rs12632942) polymorphic variant had highly significant association with clinically relevant severity (Grade 0+1 vs Grade 2+3) of chronic OXAIPN (Table 4) when compared to wild type allele A (P=0.006, OR=2.0, 95% CI=1.2 - 3.3). These findings indicate that SCN9A rs6754031 variant allele protects against both the development and severity of OXAIPN while other SCN9A rs6746030, SCN10A rs12632942 polymorphic variants were associated with either the development of chronic OXAIPN or severity of chronic OXAIPN (Table 5).

SCN4A and SCN5A variants were neither associated with the development of chronic OXAIPN nor severity of chronic OXAIPN in the current study cohort. When we did subgroup analysis with patients who have received a median dose ≥780 mg/m² of oxaliplatin, rs2302237 variant of SCN4A in addition to SCN9A (rs6754031) and SN10A (rs12632942) has exhibited statistical significance with severity of chronic OXAIPN (Table 6).

Discussion

OXAIPN affects a considerable number of patients during the therapy with OXA and can lead to postponement of therapy temporarily or complete stoppage of the therapy (Baek et al., 2010; Lehky et al., 2004). Maximum number of patients recover from the OXAIPN upon a variable period of time but quality of life is affected in these patients due to nerve damage on long term (Argyriou et al., 2008; Padman et al., 2015). To date several studies have attempted to explain the mechanism of OXAIPN, but the molecular bases for this OXAIPN remain unclear.

The proposed pathogenesis for cumulative chronic OXAIPN is the decline in cellular metabolism and axoplasmic transport due to the drug OXA accumulation in dorsal root ganglia (DRG) cells, together with oxidative stress and mitochondrial dysfunction, thus producing apoptosis of DRG neuron (Argyriou et al., 2012; Cavaletti et al., 2011). Type of chemotherapy, cumulative dose, time of infusion, and the pre-existence of peripheral neuropathy prior to the infusion of OXA are the most common risk factors for the genesis of OXAIPN (Argyriou et al., 2013b; Grothey, 2005). Even though, a number of studies have identified risk factors for the development of OXAIPN but there is lack of a reliable or specific molecular genetic biomarker to determine a causal relationship with the development of chronic OXAIPN (Cavaletti et al., 2011; McWhinney et al., 2009).

Several pharmacogenetic studies have attempted to find the polymorphisms that contribute to the disparity in susceptibility to OXAIPN (Custodio et al., 2014; McWhinney et al., 2009). Among those studies, Lecomte et al study found a significant correlation between the glutathione S-transferase p1 (GSTP1) polymorphism and chronic OXAIPN (Lecomte et al., 2006) but results from other studies reported contradicting findings with regarding the association of GSTP1 with OXAIPN (Kweekel et al., 2009; Ruzzo et al., 2007). Most of the studies have focused on a limited number of candidate genes.
| Gene and SNP | Total number of patients | OR (95%CI) | p value | Incidence of OXAIIPN | p value |
|--------------|--------------------------|------------|---------|----------------------|---------|
| SCN4A– C˃T rs2302237 | | | | | |
| CC | 114 (82.6) | 39 (86.6) | 44 (93.6) | 31 (68.9) | 0 (0.0) |
| CT | 20 (14.5) | 5 (11.1) | 3 (6.4) | 11 (24.4) | 1 (100.0) |
| TT | 4 (2.9) | 1 (2.2) | 0 (0.0) | 3 (6.7) | 0 (0.0) |
| SCN5A-C ˃G rs11720524 | | | | | |
| CC | 95 (68.8) | 31 (68.8) | 30 (63.8) | 34 (75.6) | 0 (0.0) |
| CG | 38 (27.5) | 11 (24.5) | 15 (31.9) | 11 (24.4) | 1 (100.0) |
| GG | 5 (3.7) | 3 (6.7) | 2 (4.3) | 0 | 0 (0.0) |
| CT vs CC+TT | 1.53 (0.52-4.53) | 0.5 | 3.7 (1.39-9.8) | 0.01* |
| CC+CT vs TT | 0.6 (0.06-6.7) | 0.7 | 0.1 (0.01-1.5) | 0.2 |
| SCN9A- G˃A rs6746030 | | | | | |
| GG | 97 (70.3) | 30 (66.7) | 34 (72.3) | 32 (71.1) | 1 (100.0) |
| GA | 41 (29.7) | 15 (33.3) | 13 (27.7) | 13 (28.9) | 0 (0.0) |
| AA | | | | | |
| GG vs AG+AA | 0.9 (0.4-2.1) | 0.9 | 1.4 (0.6-6.31) | 0.4 |
| GG+AG vs AA | | | | |
| GA vs GG+AA | 1.26 (0.56-2.8) | 0.7 | 0.84 (0.40-1.99) | 0.9 |
| SCN9A- T˃G rs6754031 | | | | | |
| TT | 61 (44.2) | 13 (28.9) | 18 (38.3) | 29 (64.4) | 1 (100.0) |
| TG | 56 (40.6) | 24 (53.3) | 23 (48.9) | 9 (20.0) | 0 (0.0) |
| GG | 21 (15.2) | 8 (17.8) | 6 (12.8) | 7 (15.6) | 0 (0.0) |
| TG+GG vs TT | 0.38 (0.17-0.81) | 0.01 | 0.27 (0.12-0.57) | 0.0009* |
| TT+TG vs GG | | | | |
| TG vs TT+GG | 0.45 (0.22-0.94) | 0.05 | 0.23 (0.1-0.53) | 0.007* |

Table 6. Association between selected SNPs of SCNAs and Chronic OXAIIPN in Patients who Received a Median Dose ≥780 mg/m² of Oxaliplatin (N=138)
The present study evaluated whether there is an association of development and severity of chronic oxaliplatin-induced peripheral neuropathy (OXAIPN) with various sodium channel polymorphic variants using different genetic models like codominant, dominant, recessive and additive model. Our study is the first study to explore the association between the sodium channel genes and chronic OXAIPN in all the possible genetic models which are available in literature. Our study results have shown that the presence of polymorphisms in sodium channel genes like SCN4A (rs2302237), SCN9A (rs6746030) and SCN10A (rs12632942) predicts either the occurrence or the severity of chronic OXAIPN in gastrointestinal cancer patients receiving OXA based chemotherapy while patients carrying SCN9A rs6754031 variant allele have the lower chances of the development of severe chronic OXAIPN.

In a prospective study, different sodium channel polymorphic variants were tested for susceptibility of OXAIPN development. The study results have shown that the presence of mutation in SCN4A (rs2302237) and SCN10A (rs12632942) predicts the development of acute OXAIPN (Argyriou et al., 2013a). In another recent study, various single nucleotide polymorphisms in SCN9A gene were tested for the development of chronic neuropathy. The study results showed that patients carrying SCN9A rs6746030 polymorphic variant had lower incidence of OXAIPN (Sereno et al., 2017). Similarly, in another prospective study, carriers of SCN9A rs6754031 polymorphic variant had higher chance of development of fibromyalgia (Vargas-Alarcon et al., 2012).

To date there is limited data regarding the influence of voltage gated sodium channel polymorphisms with occurrence or severity of chronic OXAIPN. This study evaluated 5 polymorphisms in 4 sodium channel genes thought to be associated with various neurological diseases. Out of 5 polymorphisms 3 SNPs have been associated either with occurrence and severity of chronic neuropathy or protection against the development of chronic OXAIPN.

To the best of our knowledge, this study is the first and the largest prospective pharmacogenetics evaluation study that tested the genetic susceptibility of selected sodium channels in a homogenous cohort of south Indian GIT cancer patients. Candidate SNPs were selected on the basis of previous published data from retrospective or single arm studies. The present study did not check gene duplications and copy number variations correlated with the sodium channel genes. Hence a genome-wide association study with large patient sample size will help in finding out the range of impact of various pharmacogenetics factors. The present study results have shown that the presence of polymorphisms in sodium channel genes predicts both the occurrence and severity of chronic OXAIPN in south Indian GIT cancer patients. Our study results have shown that patients carrying sodium channel genes like SCN4A (rs2302237) and SCN10A (rs12632942) have higher chances of development of severe chronic OXAIPN. However, further studies from independent groups are warranted to validate these results.

### Table 6. Continued

| Gene and SNP | Total number of patients | Grade 0 (N=138) | Grade 1 (N=45) | Grade 2 (N=47) | Grade 3 (N=45) |
|--------------|-------------------------|----------------|----------------|----------------|----------------|
| SCN10- A˃G (rs12632942) | AA | 82 (59.4) | 31 (68.9) | 30 (63.8) | 20 (44.4) | 1 (100.0) |
|              | AG | 53 (38.4) | 14 (31.1) | 17 (36.2) | 22 (48.9) | 0 (0.0) |
|              | GG | 3 (2.2) | 0 | 0 | 3 (6.7) | 0 (0.0) |
|              | OR (95%CI) | 0.5 (0.2-1.2) | 0.1 |
|              | p value | 0.2 |
| SCN10- A˃G (rs12632942) | OR (95%CI) | 1.82 (0.85-3.86) | 0.1 |
|              | p value | 0.1 |
| SCN10- A˃G (rs12632942) | OR (95%CI) | 2.34 (1.13-3.39) | 0.03* |
|              | p value | 0.03* |
| SCN10- A˃G (rs12632942) | OR (95%CI) | 1.59 (0.75-3.39) | 0.2 |
|              | p value | 0.2 |

* p value < 0.05 is statistically significant; OR, Odd Ratio; 95%CI, 95% Confidence Interval
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