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Effects of genetic variation in organic cation transporters (OCT) on oxaliplatin-induced neurotoxicity

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Abstract

Background: Although the addition of oxaliplatin to fluoropyrimidine treatment leads to increased progression free survival in metastatic colorectal cancer (mCRC), its use is hampered by the frequent occurrence of peripheral sensory neuropathy. Based on pre-clinical studies, it has been assumed that organic cation transports (OCTs) are involved in the neurotoxic effects of oxaliplatin. We hypothesized that polymorphisms in the genes encoding for OCT1 and OCT2, as well as for the human multidrug and compound extrusion protein 1 (hMATE1), influence the incidence and severity of oxaliplatin-induced neurotoxicity.

Methods: Patients with mCRC were recruited from the CAIRO2 study, a multicenter trial randomizing between first-line treatment with capecitabine-oxaliplatin-bevacizumab (CAPOX-B) versus CAPOX-B plus cetuximab. Patients were divided into three phenotype groups, dependent on the extent of neurotoxicity. A total of nine SNPs in *SLC22A1, SLC22A2* and *SLC47A1,* encoding for OCT1, OCT2 and hMATE1, respectively, were selected based on literature search. Genotyping was performed on germline DNA, derived from EDTA-blood samples collected at baseline.

Results: 419 patients who completed at least 4 cycles of oxaliplatin and had available clinical and genotyping data were included in the analyses. We found *SLC22A1* Arg61Cys univariately and multivariately associated with neurotoxicity phenotype, with a protective effect for homozygote variant genotype carriers. None of the other selected markers showed an association with neurotoxicity in our patient group.

Conclusion: *SLC22A1* Arg61Cys is a potential predictive marker for decreased risk of oxaliplatin-induced neurotoxicity in mCRC patients. This marker may select a group of patients capable of tolerating a higher dose of oxaliplatin, or with a lower risk of neurotoxicity at standard dose.

Introduction

First-line treatment for metastatic colorectal cancer (mCRC) often consists of a fluoropyrimidine and oxaliplatin, combined with bevacizumab. In the adjuvant setting, oxaliplatin was introduced for the combination with 5-FU, after publication of the MOSAIC-trial.[1] Oxaliplatin was shown to have synergistic effects when combined with 5-FU[2], and its addition to fluoropyrimidine-therapy in standard first-line treatment of mCRC has resulted in an improvement of progression free survival.[3] However, treatment is hampered by the development of peripheral sensory neuropathy, which can persist for months to years after the last dose of oxaliplatin or even throughout life.[4] This can have a substantial effect on quality of life with persistent handicaps, including pain and loss of sensory or motor nerve function. Phase I studies of oxaliplatin showed that neurotoxicity is the main dose-limiting side effect.[5] Many strategies have been applied in an attempt to reduce its incidence, including altered infusion schedules[6], administration of carbamazepine[7], and calcium/magnesium infusions.[8] However, chronic neurotoxicity remains an important cause of dose-modifications and treatment discontinuation in patients treated with oxaliplatin.

In current practice, it cannot be reliably predicted which patients will experience severe neuropathic side-effects. Clinical patient characteristics have been associated with increased neurotoxicity, such as anemia, hypoalbuminemia and alcohol consumption.[9] The interpatient variation may also be explained by differences in metabolism, cellular uptake and excretion of oxaliplatin, or by a different effect on neuronal cellular components. Genetic variants in genes encoding for oxaliplatin metabolizing enzymes (such as *GSTP1*[10;11] and *AGXT*[12]), for membrane efflux proteins (such as *ABCC2*[12]) and for voltage-gated sodium channels (such as *SCN4A* and *SCN10A*[13]) have been evaluated for their effect on oxaliplatin-induced neurotoxicity, with varying results.

Organic cation transporters type 1 and 2 (OCT 1 and OCT2, respectively) are involved in the cellular uptake of platinum-compounds. OCT1/OCT2 negative cells show impaired uptake of oxaliplatin, as well as increased cell survival upon incubation with oxaliplatin, compared to OCT transfected cells.[14] Pre-clinical studies showed that the accumulation of platinum is higher in dorsal root ganglia than most other tissues.[15;16] OCTs have been detected in dorsal root ganglia, and are therefore potential determinants of oxaliplatin-induced neurotoxicity.[17] In addition, the human multidrug and compound extrusion protein 1 (hMATE1) appears to play a functional role in cellular uptake of oxaliplatin.[18]

Genetic variation in the genes encoding for these cellular transporters may interfere with oxaliplatin uptake, and therefore influence its cytotoxic effects. Multiple single nucleotide polymorphisms (SNPs) have been described for each of these genes. In this clinical association study, we evaluated the effect of SNPs in the genes encoding for OCT1 (*SLC22A1*), OCT2 (*SLC22A2*) and hMATE1 (*SLC47A1*) on the incidence and severity of oxaliplatin-induced neurotoxicity in mCRC patients treated with oxaliplatin-containing chemotherapy.

Patients and methods

Patients for this clinical association study were recruited from the CAIRO2 trial[19], a multicenter phase III trial of the Dutch Colorectal Cancer Group (DCCG), which randomized between first-line treatment for mCRC with capecitabine-oxaliplatin-bevacizumab (CAPOX-B) versus CAPOX-B plus cetuximab. Patients were enrolled between June 2005 and December 2006 in 79 hospitals across the Netherlands. All patients received capecitabine 1000mg/m^2 b.i.d. orally on days 1-14 in a three weekly cycle. Oxaliplatin 130mg/m2 was administered intravenously on day 1 of each treatment cycle, with a maximum of 6 treatment cycles. Bevacizumab 7.5mg/m^2 was administered intravenously on day 1 of each cycle. For patients randomized to the CAPOX-B plus cetuximab group, cetuximab was administered intravenously at a loading dose of 400mg/m^2 on the first treatment day, followed by 250mg/m^2 once weekly thereafter. Treatment was continued until disease progression, unacceptable toxicity or death, whichever occurred first.

Patient eligibility criteria and guidelines for response assessment in the CAIRO2 trial have been described in detail elsewhere.[19] The trial protocol provided guidelines for dose-modifications in case of serious toxicity. In case of persistent paresthesia, temporary painful paresthesia or functional impairment, a 25% dose reduction of oxaliplatin was ordered. If painful paresthesia or functional impairment persisted for more than two weeks, oxaliplatin was omitted until recovery and was then initiated again at a reduced dose of 50% of the initial dose. If despite 50% dose reduction neurotoxicity recurred, patients went off-study. In case of dose reduction, dose delay, or discontinuation of treatment, the reason for the adjustment was noted in the patient file.

All included patients gave written informed consent before inclusion for the main study and the pharmacogenetic side study.

SNP selection and genotyping

A Pubmed literature search was performed to find relevant citations of SNPs in *SLC22A1*, *SLC22A2*, and *SLC47A1*. Keywords used were: (oxaliplatin, or platinum); ("organic cation transporter", or OCT; OCT1, or SLC22A1); (OCT2, or SLC22A2); (MATE1, or hMATE1, or "multidrug and toxin extrusion", or SLC47A1); (pharmacogenetics, or pharmacogenomics, or polymorphism, or SNP, or mutation).

SNPs with a minimum of three citations in Pubmed, of which at least one had to report on functional effects, were evaluated for selection. This criterion was adopted to limit the SNP selection to established markers. Only polymorphisms with a minor allele frequency (MAF) in Caucasians of 0.04 or higher were considered for inclusion. This led to the selection of four SNPs in *SLC22A1* (rs34059508, rs12208357, rs35167514, rs628031), two SNPs in *SLC22A2* (rs316019, rs145405955), and one SNP in *SLC47A1* (rs2289669).

In addition, three SNPs in *SLC47A1* (rs77630697, rs76645859, rs35395280) were selected. Although these SNPs did not fulfill the pre-set criteria, they were nonetheless included, because

they were specifically reported to influence cellular oxaliplatin uptake in a recent report.[18]

Peripheral EDTA-blood samples were collected at baseline and germline DNA was extracted with the Magnapure LC (Roche Diagnostics, Almere, The Netherlands) according to manufacturer's instructions. An Open Array technique (Lifetechnologies) was applied for genotyping of all SNPs, using predesigned assays for rs34059508 (*SLC22A1* Gly465Arg), rs12208357 (*SLC22A1* Arg61Cys), rs628031 (*SLC22A1* Met408Val), rs316019 (*SLC22A2* Ala270Ser), rs2289669 (*SLC47A1intronic*), and rs3595280 (*SLC47A1* Cys497Phe). Custom assays were designed for the genotyping of rs145450955 (*SLC22A2* Thr201Met), rs76645859 (*SLC47A1* Val480Met), rs77630697 (*SLC47A1* Gly64Asp). Unfortunately, no assay could be designed for rs35167514 (*SLC22A1* Met420del) for this technique, and this marker was therefore not included in the analyses. Negative controls (water), as well as positive controls with known genotypes were included in all runs. For further quality control, a proportion of the samples was analysed in duplicate. Because of technical difficulties, more samples were re-analysed than initially planned. In total, 280 analyses were performed in duplicate, with no inconsistencies. Samples in which five or more SNPs failed to genotype were excluded from the analyses.

Statistical analyses

Toxicity was assessed at each visit by taking the patients history, physical examination, and hematology and biochemical laboratory tests. Toxic effects were classified following the US National Cancer Institute Common Toxicity Criteria (CTC), version 2.0. Neurotoxicity was scored based on patient report only.

Symptoms of neurotoxicity were only included if developed during the first 6 courses of chemotherapy (i.e. during administration of oxaliplatin). For statistical reasons, the extent of neuropathic symptoms was categorized in three distinct phenotype groups: no neurotoxicity (patients who did not experience peripheral sensory neuropathy during any of the first 6 treatment cycles), severe neurotoxicity (patients who either experienced grade 3 neuropathy, or who had oxaliplatin dose reduction or treatment discontinuation because of neuropathy) and intermediate phenotype (all other patients). The development of oxaliplatin-induced neurotoxicity is thought to be dependent on cumulative dosage.[20] Therefore, patients with early treatment discontinuation (i.e. during the first three cycles), whether due to adverse events, disease progression or other factors, were excluded from the analyses.

Association of genotype with neurotoxicity phenotype group was then assessed by crosstabulation and the Chi-squared test. An additive effect of variant alleles was assumed. A significance threshold of $P<0.05$ was adopted for this exploratory study. Multivariate linear regression analysis was performed for the association of genotype with neurotoxicity phenotype, including cumulative dose of oxaliplatin per square meter, treatment arm and age at inclusion as covariates.

Genotype distributions were tested for agreement with those expected under Hardy-Weinberg equilibrium (HWE) using the Chi-squared test, with a threshold of $P<0.05$. Because

the selection of patients who completed a minimum of four cycles of oxaliplatin may have inadvertently led to the selection of patients with a specific genotype, adherence to HWE was calculated in the total population of patients for whom genotyping was successful for at least half of the SNPs.

All analyses were performed using SPSS version 20 software (IBM Corp., Armonk, New York, USA).

Results

Patient characteristics

In the CAIRO2 study, in total 755 patients were randomized between first-line treatment with CAPOX-B or CAPOX-B with cetuximab. Four hundred and nineteen patients who completed a minimum of four cycles of oxaliplatin and had successful genotyping for at least five of the selected SNPs were included in the analyses.(Figure 1)

Baseline characteristics for the included patients are shown in Table 1. Distributions across baseline characteristics were similar for the patients included in the pharmacogenetics analyses, compared to the total CAIRO2 population.[19]

	PGx analyses population N = 419	**CAIRO2 population[19]**
Age in years median (range)	61.3 (27.6-83.6)	62 (27-84)
Gender		
Male	249 (59.4%)	59.5%
Female	170 (40.6%)	40.5%
Treatment arm		
CAPOX-B	214 (51.1%)	50.0%
CAPOX-B plus C	205 (48.9%)	50.0%
LDH at baseline		
Normal	234 (55.8%)	56.7%
Above ULN	185 (44.2%)	43.3%
PFS		
0	263 (62.8%)	62.5%
1	150 (35.8)	37.5%
unknown	6 (1.4%)	
Prior chemotherapy		
No	361 (86.2%)	84.9%
Yes	58 (13.8%)	15.1%

Table 1. Baseline characteristics

CAPOX-B, capecitabine, oxaliplatin and bevacizumab; CAPOX-B plus C, capecitabine, oxaliplatin and bevacizumab plus cetuximab; LDH lactate dehydrogenase; PFS, performance score; PGx, pharmacogenetics; ULN, upper limit of normal.

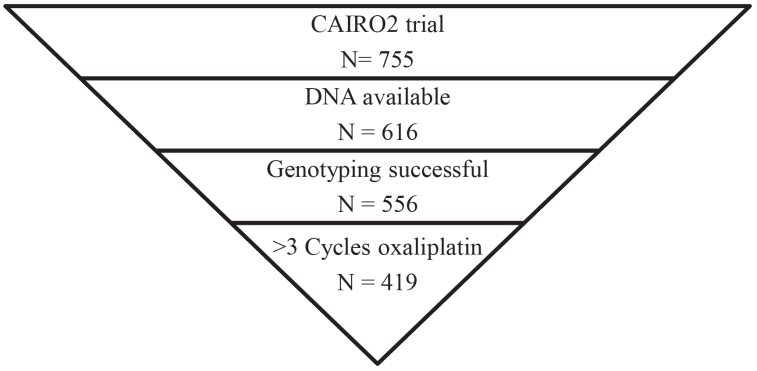

Figure 1. Schematic presentation of patient numbers included in pharmacogenetic analyses

Association of clinical parameters with neurotoxicity

Patients included in the pharmacogenetic analyses were separated into three phenotype groups, as described in the methods section. We found 47 (11.2%) patients experienced no neurotoxicity during the first six courses of chemotherapy, 66 (15.8%) patients had severe neurotoxicity, and the remaining 306 (73.0%) patients had an intermediate phenotype.

Cumulative doses of oxaliplatin, expressed as milligrams per square meter of body surface area (BSA) were calculated for all included patients and rounded to the nearest ten. Mean cumulative doses of oxaliplatin were significantly lower for patients with severe neurotoxicity (688 mg/m^2, 95% confidence interval [95% CI] 644-691mg/m^2), than for patients without neurotoxicity (727 mg/m^2, 95% CI 701-754mg/m^2), and for those with the intermediate phenotype (739 mg/m^2, 95% CI 730-749mg/m^2) (Kruskal-Wallis test, P = *0.000*). Consistent with these results, a significantly higher number of patients with the severe neurotoxicity phenotype had a dose reduction of oxaliplatin in any cycle (50/66, 92%), compared to patients with either no neurotoxicity (6/47, 13%) or the intermediate phenotype (54/306, 18%) (Chi-square test, P = *0.000*). However, there were no differences between the phenotype groups in the administered number of cycles of oxaliplatin per patient, or in delays in oxaliplatin-administration (data not shown).

Patients with severe neurotoxicity had a slightly higher mean age (63.8 years, 95% CI 61.5-66.1 years), than patients without neurotoxicity (60.7 years, 95% CI 57.9-63.4 years) or those with the intermediate phenotype (61.0 years, 95% CI 60.0-62.1 years), but the difference was non-significant (one way ANOVA, P = *0.088*). The extent of neurotoxicity was not associated with patient gender (P = *0.255*), LDH at baseline (P = *0.322*), WHO performance score (P = *0.709*) or prior adjuvant chemotherapy (P = *0.724*).

There was an overrepresentation of patients treated with CAPOX-B plus cetuximab in the "no neurotoxicity" phenotype group. In other words, patients who were treated with CAPOX-B alone had a slightly higher chance of at least intermediate phenotype neurotoxicity, compared to patients who were treated with CAPOX-B plus cetuximab (P = *0.052*).

Genotyping results

Genotyping was successful for all nine polymorphisms in 274 of the 419 included patients (65.4%). Call rates for individual polymorphisms varied from 77.1% (323/419 patients) for *SLC47A1* Cys497Phe to 99.3% (416/419 patients) for *SLC47A1* Gly46Asp.

Genotype distributions followed Hardy-Weinberg equilibrium for all selected SNPs, accept for *SLC22A1* Arg61Cys ($\chi^2 = 9.77$) and *SLC22A1* Met408Val ($\chi^2 = 6.08$), but allele frequencies are consistent with those reported in literature.[21] Four of the selected SNPs were monoallelic in our population (*SLC22A2* Thr201Met, *SLC47A1* Val480Met, *SLC47A1* Gly64Asp, and *SLC47A1* Cys497Phe). Genotype distributions for all patients in whom genotyping was successful and for those included in the pharmacogenetic analyses are described in Table 2.

Association of genotype with neurotoxicity

Polymorphic SNPs (*SLC22A1* Gly465Arg, *SLC22A1* Arg61Cys, *SLC22A1* Met408Val, *SLC22A2* Ala270Ser, and *SLC47A1* non-coding) were univariately assessed for their association with neurotoxicity phenotype. Results for these analyses are shown in Table 3.

SLC22A1 Arg61Cys (rs12208357) showed a statistically significant association with neurotoxicity phenotype. None of the patients carrying the homozygote variant genotype experienced severe neurotoxicity, compared to 8.5% of heterozygote patients and 17.8% of patients carrying the homozygote wildtype genotype (P = *0.011*).

The association of genotype with neurotoxicity phenotype was then explored in multivariate analysis, treating age, treatment arm and cumulative oxaliplatin dose per BSA as covariates. The association of *SLC22A1* Arg61Cys genotype with the extent of neurotoxicity was preserved in multivariate analysis (P multivariate = *0.015*). This effect was also seen when patients who discontinued oxaliplatin treatment during the first 3 courses were included in the analysis (P = *0.045,* data not shown). None of the other polymorphisms were associated with neurotoxicity phenotype in univariate or multivariate analysis.

	SNP number	Variation	Call rate	Total population*	PGx analyses population*
				N = 556	N = 419
SLC22A1 Gly465Arg	rs34059508	1393G>A	94.5%	0.97-0.03-0.00	0.96-0.04-0.00
SLC22A1 Arg61Cys	rs12208357	181C>T	85.2%	0.85-0.13-0.02	0.85-0.13-0.02
SLC22A1 Met408Val	rs628031	1222G>A	85.4%	0.35-0.44-0.21	0.35-0.43-0.22
SLC22A2 Ala270Ser	rs316019	808C>A	87.8%	0.80-0.18-0.02	0.80-0.18-0.02
SLC22A2 Thr201Met	rs145450955	602C>T	98.3%	1.00-0.00-0.00	1.00-0.00-0.00
SLC47A1 non-coding	rs2289669	G>A	96.4%	0.33-0.51-0.16	0.34-0.50-0.16
SLC47A1 Gly64Asp	rs77630697	191G>A	99.3%	1.00-0.00-0.00	1.00-0.00-0.00
SLC47A1 Val480Met	rs76645859	1438G>A	99.0%	1.00-0.00-0.00	1.00-0.00-0.00
SLC47A1 Cys497Phe	rs35395280	1490G>C	77.1%	1.00-0.00-0.00	1.00-0.00-0.00

Table 2. Genotype distributions

SNP, Single nucleotide polymorphism; PGx, pharmacogenetics.
* Genotype distributions are shown as: homozygote wildtype – heterozygote – homozygote variant type.

		Neurotoxicity phenotype				
		No neurotoxicity	Intermediate phenotype	Severe neurotoxicity	P-value univariate	P-value multivariate
SLC22A1 Gly465Arg	GG	11.0%	73.0%	16.0%	*0.365*	*0.627*
	GA	21.4%	57.1%	21.4%		
SLC22A1 Arg61Cys	CC	9.5%	72.8%	17.8%		
	CT	12.8%	78.7%	8.5%	*0.011*	*0.015*
	TT	50.0%	50.0%	0.0%		
SLC22A1 Met408Val	GG	9.7%	77.4%	12.9%		
	GA	9.0%	75.0%	16.0%	*0.728*	*0.331*
	AA	11.5%	69.2%	19.2%		
SLC22A2 Ala270Ser	CC	12.2%	72.1%	15.6%		
	CA	3.0%	80.6%	16.4%	*0.280*	*0.321*
	AA	14.3%	71.4%	14.3%		
SLC47A1 intron	GG	12.6%	70.4%	17.0%		
	GA	10.3%	73.4%	16.3%	*0.843*	*0.851*
	AA	10.6%	77.3%	12.1%		

Table 3. Univariate analyses for the association of genotype with extent of oxaliplatin-induced neurotoxicity

Discussion

Organic cation transporters are proposed determinants of oxaliplatin-induced neurotoxicity. We investigated the effect of germline genetic variation in genes encoding for OCT1 (*SLC22A1*), OCT2 (*SLC22A2*), and hMATE1 (*SLC47A1*) on the incidence and severity of neurotoxicity in 419 mCRC patients treated with oxaliplatin-containing chemotherapy. We found that *SLC22A1* Arg61Cys was significantly associated with the extent of oxaliplatin-induced peripheral sensory neuropathy, with a protective effect for carriers of the homozygote variant genotype. None of the other selected polymorphisms showed an association with neurotoxicity in our study population.

To our knowledge, this is the first study to address the effect of polymorphisms in these genes on oxaliplatin-induced neurotoxicity. Pharmacogenetic research so far has led to the identification of other potential predictors for neurotoxicity. Polymorphisms in the gene encoding for the metabolic enzyme alanine glyoxylate transferase (*AGXT)*[12], as well as variation in *SCN4A* and *SCN10A*, encoding for voltage gated sodium channels[13], have been associated with the incidence of oxaliplatin-induced neuropathy. Earlier, germline genetic variation in the detoxifying enzyme glutathione S-transferase π1 (*GSTP1*), was identified as a possible predictor of neurotoxicity caused by oxaliplatin[22], but this result could not be replicated by our own studygroup.[10] Additionally, a genome wide association study in Korean colorectal cancer patients identified five SNPs that were not previously associated with oxaliplatin-induced neurotoxicity.[23] However, all of these results have not been sufficiently validated, and cannot be incorporated into clinical practice at present.

Based on the assumptions that peripheral neuropathy is an important complication of oxaliplatin-treatment[5;20], that platinum-uptake is increased in dorsal root ganglia[15;16], and that OCT2 is present as a transporter in dorsal root ganglia[17], we proposed that the influence of OCT-function on oxaliplatin-induced neurotoxicity should be further evaluated. Indeed, OCT1 and -2, as well as hMATE1, are involved in transportation of oxaliplatin into cells expressing these transporters[24], and OCT-transfected cells are over 20 times more sensitive to oxaliplatin than empty-vector cells.[14] Likewise, cells completely lacking hMATE1 are more chemoresistent to oxaliplatin than hMATE1 positive cells.[18] Noteworthy, colorectal cancer cells express a high level of organic cation transporter type 3 (OCT3)[25], but not OCT2[17], which may explain the lack of correlation between anti-cancer effect and the development of neuropathy on oxaliplatin-based chemotherapy.

In vivo experiments showed that, after a single administration of oxaliplatin, OCT1/2 positive mice experienced increased sensitivity to cold and mechanical stimulation, compared to OCT1/2 knock-out mice[17]. Concurrent administration of cimetidine, a known competitive inhibitor of OCT1 and -2[14], resulted in complete protection from cold-sensitivity in OCT1/2 positive mice.[17] These preclinical results confirm the relationship between OCT1/2 function and oxaliplatin-induced neuropathy.

A multitude of germline genetic polymorphisms have been described for OCT1, OCT2 and hMATE1, and selected variants have shown functional effects on compound transport.[26-29] We hypothesized that functional polymorphisms in *SLC22A1*, *SLC22A2* and *SLC47A1* are associated with the incidence and severity of oxaliplatin-induced neurotoxicity. Indeed, *SLC22A1* Arg61Cys is a missense variant located in exon 1, on the first large loop of the protein, and the residue change results in reduced mRNA expression and loss of OCT protein function.[21;30-36] Our results, showing a protective effect of the Cys/Cys homozygote variant genotype on neuropathic complaints after oxaliplatin-administration, are in line with the aforementioned data. This SNP may therefore be a predictive marker for a decreased risk of oxaliplatin-induced neurotoxicity. Since neurotoxicity is the main dose-limiting event in oxaliplatin-treatment, patients carrying the protective genotype may actually be able to endure a higher dose of oxaliplatin than determined in phase I trials.

Despite this promising result, our study is subject to several difficulties, both in clinical and technical aspects. Oxaliplatin-induced neurotoxicity appears in two distinct forms: acute and chronic neuropathy. Acute neuropathy occurs in the vast majority of patients, during hours or days after infusion of oxaliplatin.[12] It is characterized by dysesthesias and paresthesias in the oropharyngeal region and extremities, which are induced or aggravated by cold.[37] These sensory symptoms are presumably caused by neuromyotonic discharges, consistent with peripheral nerve hyperexcitability.[7] In contrast, chronic neurotoxicity develops in the course of treatment. It was originally thought to occur in less than a quarter of patients[12], and only after a cumulative dosage of 500mg/m2 oxaliplatin.[20] However, newer studies have shown that almost 85 percent of patients treated with oxaliplatin experience some degree of chronic neurotoxicity.[38] This phenomenon is caused by progressive loss of sensory fibers, thereby

inducing sensory axonal neuropathy.[39] There appears to be a correlation between the amount of acute neuropathic symptoms and the severity of chronic oxaliplatin-induced neuropathy.[40] Different scoring systems are used for assessing the intensity of oxaliplatin-induced neuropathy. Most studies use the National Cancer Institute-Common Toxicity Criteria (NCI-CTC). An oxaliplatin-specific scale has been described by Levi et al[41], which takes into account the duration of symptoms, as well as the intensity. In our patient population, no distinction was made between acute and chronic neuropathy.

As described above, it is suggested that there is a difference in the etiology of chronic versus acute oxaliplatin-induced neurotoxicity.[7;39] Pre-clinical studies so far have only shown an effect of OCT2 on acute neurotoxicity in mice.[17] No data are available for OCT effects on chronic oxaliplatin-induced neurotoxicity. Our results may therefore have been clouded by the inclusion of patients in whom neurotoxic symptoms, whether acute or chronic, were mediated by systems other than OCT.

Another clinical pitfall is our lack of complete information on other determinants of neurotoxicity, such as alcohol consumption or co-medication. For instance, uptake efficacy of hMATE1 is influenced by many other drugs, such as omeprazole and antibiotics.[42] Interestingly, its transport capacity may also be inhibited by irinotecan.[43] If any of the co-administered medications impair oxaliplatin uptake by OCT1, OCT2 or hMATE1, they may have lowered the incidence of neurotoxicity in patients taking these drugs independent of the investigated genotypes, thereby obscuring our results.

Technical difficulties may also weaken the validity of our results. Genotyping failed for five or more SNPs in 60 patients, almost 10% of our population. Even after elimination of these samples, genotyping was successful for all markers in less than half of the patients. The exclusion of samples based on missing genotypes may have induced bias in our analyses. We also excluded patients who completed only 1 to 3 cycles of oxaliplatin. However, baseline-characteristics in the selected population are similar to those for the total CAIRO2 population (Table 1), and allele frequencies are comparable between the selected population and all genotyped patients.(Table 2) We therefore believe this clinical selection did not confound the analyses.

We found that four out of nine SNPs were monoallelic in our population. Frequency data on these SNPs in all ethnicities are scarce and minor allele frequencies may differ between populations.[21] In addition, *SLC22A1* Arg61Cys is not in strong linkage disequilibrium with the other selected SNPs in Caucasians, but a haplotype block including this SNP and *SLC22A1* Met408Val was identified in Asian subjects.[26;44] It is therefore not certain that results for *SLC22A1* Arg61Cys can be extrapolated to other populations, or that this is the best predicting SNP in the chromosomal region.

Although the evidence supporting our hypothesis seems solid, there are some inconsistencies. Whereas one study found both OCT1 and OCT2 are involved in cellular uptake and cytotoxicity of oxaliplatin[14], others found that OCT2, but not OCT1 was essential for oxaliplatin transport into the cell.[17;24;45] Furthermore, contrasting results have been published

for the effect of the selected polymorphisms on OCT function. Whereas some studies report on the cellular uptake of different substances[46-48], others have used more elaborate endpoints, such as drug efficacy or renal clearance.[49-53] This may in part explain the contradicting results.

In conclusion, in our population of mCRC patients treated with CAPOX-B either with or without cetuximab, a SNP in the gene encoding for OCT1 (*SLC22A1* Arg61Cys) associated with the absence of severe oxaliplatin-induced neurotoxicity. This result needs to be validated in independent patient cohorts. In these validation studies, neurotoxicity should be assessed by an oxaliplatin-specific scale, or physical diagnostic tests, to better grade the extent of sensory neuropathy and to distinguish acute from chronic neurotoxicity. Because of the potential interaction with other drugs, treatment regimen should ideally be uniform across all patients and co-medication meticulously monitored.

Because peripheral sensory neuropathy is the main dose-limiting toxicity in oxaliplatin-based chemotherapy, this SNP may select a group of patients capable of tolerating a higher than average dose of oxaliplatin, perhaps leading to an increment in treatment efficacy.

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