Open access • Journal Article • DOI:10.1158/1078-0432.CCR-10-1741

A polymorphism in the cytidine deaminase promoter predicts severe capecitabine-induced hand-foot syndrome — Source link

Daniela Caronia, Miguel Martin, Javier Sastre, Julio de la Torre ...+7 more authors

Published on: 01 Apr 2011 - Clinical Cancer Research (American Association for Cancer Research)

Topics: Capecitabine, Cytidine deaminase and Thymidine phosphorylase

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

Clinical Cancer Research

A Polymorphism in the *Cytidine Deaminase* Promoter Predicts Severe Capecitabine-Induced Hand-Foot Syndrome

Daniela Caronia, Miguel Martin, Javier Sastre, et al.

Clin Cancer Res 2011;17:2006-2013. Published OnlineFirst February 16, 2011.

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

Clinical Cancer Research

Predictive Biomarkers and Personalized Medicine

A Polymorphism in the *Cytidine Deaminase* Promoter Predicts Severe Capecitabine-Induced Hand-Foot Syndrome

Daniela Caronia[1], Miguel Martin[2], Javier Sastre[3], Julio de la Torre[3], José Angel García-Sáenz[3], Maria R. Alonso[1], Leticia T. Moreno[1], Guillermo Pita[1], Eduardo Díaz-Rubio[3], Javier Benítez[1,4], and Anna González-Neira[1]

Abstract

Purpose: Hand-foot syndrome (HFS) is one of the most relevant dose-limiting adverse effects of capecitabine, an oral prodrug of 5-fluorouracil used in the standard treatment of breast and colorectal cancer. We investigated the association between grade 3 HFS and genetic variations in genes involved in capecitabine metabolism.

Experimental Design: We genotyped a total of 13 polymorphisms in the carboxylesterase 2 (*CES2*) gene, the cytidine deaminase (*CDD*) gene, the thymidine phosphorylase (*TP*) gene, the thymidylate synthase (*TS*) gene, and the dihydropyrimidine dehydrogenase (*DPD*) gene in 130 patients treated with capecitabine. We correlated these polymorphisms with susceptibility to HFS.

Results: We found an association of HFS appearance with rs532545 located in the promoter region of *CDD* (OR = 2.02, 95% CI = 1.02–3.99, $P = 0.039$). Because we found no association between the *rs532545* genotype and CDD mRNA expression in Epstein-Barr virus lymphoblastoid cells, we explored additional genetic variations across the *CDD* promoter. We found an insertion, rs3215400, in linkage disequilibrium with rs532545 ($D' = 0.92$), which was more clearly associated with HFS (OR = 0.51, 95% CI = 0.27–0.95, $P = 0.028$) in patients and with total *CDD* gene expression ($P = 0.004$) in lymphoblastoid cells. *In silico* analysis suggested that this insertion might create a binding site for the transcriptional regulator E2F. Using a SNaPshot assay in lymphoblastoid cells, we observed a 5.7-fold increased allele-specific mRNA expression from the deleted allele.

Conclusions: The deleted allele of rs3215400 shows an increased allele-specific expression and is significantly associated with an increased risk of capecitabine-induced HFS. *Clin Cancer Res; 17(7); 2006–13. ©2011 AACR.*

Introduction

Capecitabine (Xeloda) is a 5-fluorouracil (5-FU) prodrug widely used in the treatment of breast and colorectal cancer (1). The conversion takes place through a 3-step enzymatic process: after activation by carboxylesterase 2 (CES2) and cytidine deaminase (CDD), capecitabine is converted to 5-FU by thymidine phosphorylase (TP), which is highly expressed in tumors and liver. One of the main targets of 5-FU is TS (thymidylate synthase). Finally, 5-FU is catabolized by DPD (dihydropyrimidine dehydrogenase; ref. 2).

Capecitabine offers a more selective alternative to 5-FU, as it is converted into the active form specifically in the tumor cell (2), lowering the adverse effects related to 5-FU (3). Even so, hand-foot syndrome (HFS) occurs in a large percentage (almost 30%) of capecitabine-treated patients. HFS is a side effect of continuous 5-FU infusion and of other drugs such as doxorubicin, cytarabine, and docetaxel (4). It is characterized by tenderness, redness, and swelling of palms and soles and often necessitates dose reduction or even treatment interruption (5).

Pharmacogenetics aims to understand the relationship between genetic variation and adverse drug reaction (ADR) or treatment response. Genetic analysis of genes involved in the metabolism of anticancer drugs is becoming more and more important for elucidating the interpatient pharmacodynamic variability of anticancer drugs.

With regard to capecitabine, some polymorphisms such as three 28-bp repeats in the 5′ untranslated region (UTR) of the *TS* gene containing a *G>C* mutation in the second repeat, a 6-bp deletion in the 3′ region of the *TS* gene, and the inactivating mutation *IVS14+1G>A* in the *DPD* gene

Authors' Affiliations: [1]Human Genotyping Unit-CeGen, Human Cancer Genetics Programme, Spanish National Cancer Research Centre; [2]Servicio de Oncología Médica, Hospital Universitario Gregorio Marañón; [3]Servicio de Oncología Médica, Hospital Universitario San Carlos; and [4]Human Genetics Group, Human Cancer Genetics Programme, Spanish National Cancer Research Centre, Madrid, Spain

Note: D. Caronia and M. Martin have contributed equally to this study.

Corresponding Author: Anna González-Neira, Human Genotyping Unit-CeGen, Human Cancer Genetics Programme, Spanish National Cancer Research Centre, Melchor Fernández Almagro 3, Madrid 28029, Spain. Phone: 3491-2246974; Fax: 3491-2246923; E-mail: agonzalez@cnio.es

doi: 10.1158/1078-0432.CCR-10-1741

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

Translational Relevance

This article shows evidences of a role of a *CDD* genetic variant in capecitabine-induced severe hand-foot syndrome appearance, suggesting that this variant might be useful to prevent this adverse event in patients treated with this drug. These findings give new insights to elucidate the mechanisms of capecitabine-related toxicity and could help in individualizing the treatment.

(6–8) have been related to severe global toxicity appearance when ADRs such as myelosuppression, hematologic toxicity, diarrhea, and HFS are grouped.

The aim of the present study was to investigate the relationship between capecitabine-induced HFS and polymorphisms in genes of the capecitabine metabolic pathway (*CES2*, *CDD*, *TP*, and *DPD*) and target gene (*TS*).

Methods

Study subjects

This retrospective study included 130 patients with a diagnosis of breast cancer or colorectal cancer treated at the Hospital Universitario San Carlos, Madrid, Spain, between June 2005 and March 2009. The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the institution. All patients signed an informed consent. Most (72%) of the patients were diagnosed with breast cancer, whereas the remaining patients had colorectal cancer. The median age at diagnosis was 63 years (range = 28–88 years), and 112 patients (86%) were female.

Capecitabine was administered according to 2 different schedules. Colorectal cancer patients were treated with a standard regimen (1,250 mg/m^2 orally every 12 hours on days 1–14 every 3 weeks), whereas breast cancer patients were treated either with the same standard regimen or with a continuous regimen (800 mg/m^2 orally every 12 hours daily).

HFS was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 2). Grade 3 HFS was defined as skin changes with pain interfering with function. For study purposes, we used as study endpoint the maximum HFS grade experienced by the patients along the treatment, considering grade 0–2 of HFS as no or low toxicity and grade 3 as high toxicity. Other clinical data were recorded such as age, sex, capecitabine regimen, number of reductions, and hepatic metastasis (as a variable that could interfere with capecitabine metabolism; Table 1).

Table 1. Clinical characteristics of patients ($N = 130$)

	Patients	
	n	**%**
Age at diagnosis, y		
Median	63	
Range	28–88	
Sex		
Female	112	86
Male	18	14
Diagnostic		
Breast cancer	93	72
Colorectal cancer	37	28
Stage		
I	3	2
II	9	7
III	33	25
IV	85	65
Treatment setting		
Postsurgical adjuvant	39	30
First-line metastatic	26	20
Second-line metastatic	13	10
Third-line metastatic or further	52	40
Capecitabine		
Standard	104	80
Continuous	26	20
No. of capecitabine reductions		
0	59	45
1	54	42
≥2	17	13
HFS		
Grade 0	41	31
Grade 1	23	18
Grade 2	25	19
Grade 3	41	32
Hepatic metastasis		
No	78	60
Yes	51	40

Genotyping

A total of 13 polymorphisms located in 5 different genes were analyzed in the 130 breast and colorectal cancer patients. Eleven polymorphisms were located in capecitabine metabolic pathway genes. In the *CDD* gene, single nucleotide polymorphisms (SNP) rs532545 −451C > T, rs602950 −92A > G, both located in the promoter, and Lys27Gln, the coding SNP rs2072671 (9–13); in the *DPD* gene, the intronic SNP rs3918290, IVS14+1G>A, in the splice donor site flanking exon 14 that causes exon skipping and inactivation of DPD allele (7, 14–16); in the *CES2* gene, rs2241409, rs11568314, rs11568311, all intronic SNPs, and rs11075646 823C > G; in the *CES2* gene promoter and in the *TP* gene, the intronic SNP rs470119 and the coding SNPs rs11479 Ser471Leu and rs131804 Ala324Ala. The remaining 2 polymorphisms were located in the 5-FU target gene *TS*, a 28-bp double- or triple-tandem repeat, including a *G* > *C* mutation in the 5′ region and a 6-bp deletion in the 3′ region (6, 14, 17).

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

All polymorphisms have been described in the literature as possible functional variants except in the *TP* and *CES2* genes, for which, because of the lack of candidate functional SNPs, tagSNPs were selected.

Genomic DNA was extracted from peripheral blood lymphocytes by automatic DNA extraction (MagNA Pure; Roche) according to the manufacturer's protocol. DNA was quantified using PicoGreen (Invitrogen Corp.).

Genotypes were determined using the KASPar SNP genotyping system (KBioscience). Allelic discrimination was carried out using the ABI PRISM 7900 Sequence Detection System (Applied Biosystems).

For the polymorphisms in the *TS* gene, restriction fragment length polymorphisms (RFLP) and direct sequencing were used. In particular for the 5′-UTR polymorphism, a fragment containing the 28-bp repeat was amplified starting from 100 ng genomic DNA, using the following primers: 5′-TTCCCGGGTTTCCTAAGACT-3′ and 5′-TGGATCTGCCCCAGGTACT-3′. PCR products were purified by ExoSap-IT (USB Corporation) and directly sequenced using an ABI3730 DNA analyzer (Applied Biosystems). 3′-UTR variation was analyzed by RFLP as described (6).

Duplicate and negative samples were included in all assays.

Fine mapping at the *CDD* promotor

A 959-bp fragment of the promoter was amplified from 30 healthy controls, using the following primers: 5′-ATGCAGTGGTGCAATCTGAG-3′ and 5′-GTGCCCACCTTTACCTTTGA-3′. After purification, DNA fragments were sequenced as described earlier.

Lymphoblastoid cell cultures

Eighty-nine lymphoblastoid cell lines derived from the Caucasian Utah CEPH lines were purchased from the Coriell Institute for Medical Research (Camden, NJ). Lymphoblastoid cell lines were cultured in RPMI 1640 containing 15% FBS (Euroclone) and maintained in a humidified incubator at 37°C and 5% CO_2.

Genomic DNA and total RNA were extracted from exponentially growing cells, using DNAZol (MBC; Molecular Research Center, Inc.) and TRIzol (Invitrogen), respectively, according to the manufacturer's protocol. DNA was genotyped for rs532545, rs602950, rs2072671, rs3215400, and rs603412 SNPs by Kaspar Genotyping Assays as described earlier.

Gene expression assay

One microgram of RNA was reverse transcribed using an oligo$(dT)_{18}$ primer and Superscript II Reverse Transcriptase (Invitrogen) according to the manufacturer's recommendations. Real-time PCR was carried out in the 89 cell lines by using the TaqMan Gene Expression Assays (Applied Biosystems), following the manufacturer's protocol (Hs00156401_m1 probe for CDD) on an ABI PRISM 7900 Sequence Detection system (Applied Biosystems). The glyceraldehyde 3-phosphate dehydrogenase transcript level was used as a reference (Hs9999905_m1). Data were analyzed using absolute quantification on resulting C_t (cycle threshold) values generated on the sequence detection system. Serial dilutions of cDNA of a control sample were used to generate standard curves, and quantity mean (Qty mean) for each sample was calculated. Each sample was evaluated in triplicate.

Prediction of transcription factor binding sites

We carried out *in silico* prediction of putative transcription factor binding sites by using TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html, v1.3) and Jaspar (http://jaspar.cgb.ki.se/).

Allele-specific expression assay

Allele-specific mRNA expression was measured amplifying a 244-bp region around SNP rs3215400 in the 43 Caucasian lymphoblastoid cell lines heterozygous for this SNP. The following primers were used: 5′-AAAGCTGCGTACCTGAGAGC-3′ and 5′-TGACTGTAGGGGCAGTAGGC-3′.

cDNA was prepared from RNA treated with DNase I (Ambion), using Superscript II Reverse Transcriptase (Invitrogen) with random hexamers according to the manufacturer's recommendations.

Primer extension with fluorescent dideoxynucleotides was done using the SNaPshot System (Applied Biosystems), followed by capillary electrophoresis on an ABI3730 DNA analyzer (Applied Biosystems). Data were analyzed by Peak Scanner 1.0 software (Applied Biosystems).

Allele-specific expression (ASE) ratios were calculated as follows: the ratio between the 2 alleles' peak area of cDNA was divided by the same ratio for genomic DNA. Each sample was assayed using 2 independent cDNA preparations; 2 independent single base extensions were run for each cDNA preparation, giving a total of 4 replicates. The SNaPshot ASE variation value for each individual is given as the average of the 4 analyses.

Statistical analysis

The main objective of the study was to assess the correlation between SNP variations and severity of HFS (low or grades 0–2, vs. high or grade 3 + 4). Associations between SNPs and HFS were assessed using logistic regression analysis, comparing genotype frequencies in patients with low toxicity and high toxicity, and estimating ORs. Individuals who were homozygous for the most frequent allele were used as the reference group.

Sex, age (continuous), tumor type (colorectal and breast cancer), capecitabine dose (continuous), and number of dose reductions were included as covariates in multivariate logistic regression.

In addition to the model comparing the genotypes separately (codominant model), we considered log-additive, dominant, and recessive models.

Associations between gene expression and SNPs in cell lines were assessed by ANOVA when considering the 3

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

genotypes separately and Student's *t* test, assuming equal variances considering the wild-type homozygous versus the polymorphic heterozygous and homozygous genotypes. All expression data (Qty mean) were $\log_2$ transformed to obtain normally distributed data.

The R (version 2.6.0.2) and the SPSS software (version 13.0, SPSS Inc.) were used for all analyses, and the values of $P \geq 0.05$ were considered statistically significant. We estimated that at a nominal statistical significance level of 0.05, our study had 80% power to detect ORs under a dominant model of at least 3.0 for all but the less frequent SNPs (rs11568314, rs11568311, and rs11479) for which the minimum OR detectable was 6.0.

Results

The polymorphisms analyzed and the genotypic frequencies are shown in Table 2. The minor allele frequencies (MAF) were between 0.06 and 0.42. There was no evidence of departure from the Hardy–Weinberg equilibrium for any of them. Call rate was more than 99% for all samples, and duplicated samples showed an extremely high concordance (>99%). Grade 3 HFS was observed in 41 (32%) of the 130 patients treated with capecitabine.

We found a significant association with HFS only for a polymorphism in the *CDD* gene. In particular, the polymorphic T allele of rs532545 was associated with higher incidence of grade 3 HFS: the estimated OR was 2.02 ($P = 0.039$). No substantial changes were observed in this OR after adjustment for capecitabine dose, tumor type, age, and hepatic metastasis, whereas adjustment for dose reduction increased the significance of the association. No evidence of association was found for the polymorphisms in the other genes considered (Table 2).

We found only 1 patient heterozygous for the *DPD* SNP rs3918290, and this patient experienced life-threatening toxicities (severe myelosupression and mucositis). This SNP was not included in the analyses. Lethal outcome or high toxicity has been reported after treatment with 5-FU or capecitabine (8, 16, 18).

Because of the SNP associated with HFS is located in the promoter of the *CDD* gene and had been described to affect transcription by Fitzgerald and colleagues (10), we analyzed the relationship with CDD mRNA levels by quantitative real-time PCR. Because RNAs were not available for our patients, 89 lymphoblastoid cell lines from Caucasian healthy individuals were used for this purpose. After genotyping of rs532545 (MAF = 0.29) in these cell lines, we found that this SNP was not associated with a significant change in mRNA levels ($P = 0.671$).

Neither of the other 2 SNPs in the *CDD* gene analyzed (rs602950, MAF = 0.29; rs2072671, MAF = 0.36) was associated with gene expression (*P* values of 0.655 and 0.327 for rs602950 and rs2072671, respectively).

We hypothesized that rs532545 might not be the causal SNP but simply a marker SNP, so we searched by fine mapping of the promoter for other variants that showed stronger association with HFS. We sequenced a 959-bp fragment at the 5′ extreme of the *CDD* gene and found, apart from the 3 SNPs originally included in the study (rs602950, rs532545, and rs2072671), 2 more common variants already annotated in the dbSNP database (rs3215400 and rs603412). We genotyped our series of 130 patients for these 2 variants and found a statistically significant association with HFS for rs3215400 (Table 3). In particular, carriers of at least one inserted C allele of rs3215400 had lower risk of developing grade 3 HFS (OR = 0.37, $P = 0.020$) compared with individuals homozygous for the deleted allele. Further analysis was carried out for this variant, comparing grade 0 versus 3 to better discriminate the HFS phenotype. Although the sample size was greatly reduced ($N = 96$), the statistical significance was maintained ($P = 0.045$). This variant is in linkage disequilibrium (LD) with the previously associated rs532545 ($D' = 0.92$), and the LD block structure under the association interval is given in Figure 1A. To see whether the effect we observed was due to a single variant or to a combination of the 2 associated polymorphisms, rs532545 and rs3215400, we also analyzed the effect of the haplotypes on HFS. We found that the haplotypes that conferred an increased risk of developing HFS were only those that contained the deleted allele of the rs3215400 variant (data not shown).

We genotyped by the same approach the 2 polymorphisms we found after promoter sequencing, rs3215400 (MAF = 0.44) and rs603412 (MAF = 0.44), in the 89 lymphoblastoid cell lines. Again, we investigated the correlation with gene expression and found that the rs3215400 variant was associated with a significant difference in CDD mRNA levels. The median expression in cell lines with the homozygous *del-del* genotype was 3.1-fold higher than that of those with *del-C* heterozygous and *CC* homozygous genotypes ($P = 0.004$; Fig. 1B). The other *CDD* variant rs603412 was not significantly associated with gene expression ($P = 0.4$).

To elucidate whether the associated rs3215400 marker could be a putative functional variant, we carried out an *in silico* analysis with two different computer tools for the prediction of transcription factor binding sites and we found that the deleted allele of rs3215400 abrogates a binding site for the transcription factor E2F (Fig. 2A). Because of the global gene expression association and location in a putative transcriptional element, a more comprehensive analysis was carried out. Specifically, we measured ASE by SNaPshot in both genomic DNA and cDNA of the 43 cell lines heterozygous for rs3215400. As expected, the allelic ratio for genomic DNA was around 1, ranging from 0.95 to 1.6; in contrast, all samples analyzed displayed an increased allele ratio in cDNA compared with genomic DNA (Fig. 2B). In all cell lines analyzed, the ASE ratio for the deleted allele versus the C allele was more than 3, with an average value of 5.7 (range = 3.9–7.7; SD = 0.83).

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

Table 2. Genotype distribution and logistic regression analyses assessing associations of polymorphisms with grade 3 HFS

Genotype	*n* (%)		OR	95% CI	*P*
	Grade 0–2 HFS	Grade 3 HFS			
CDD rs532545					
Dominant					
CC	37 (41.6)	11 (26.8)	Referent		
CT/TT	52 (58.4)	30 (73.2)	2.28	0.95–5.44	0.057
Additive			2.02	1.02–3.99	0.039
CDD rs602950					
Dominant					
AA	37 (41.6)	13 (31.7)	Referent		
AG/GG	52 (58.4)	28 (68.3)	1.82	0.79–4.22	0.153
Additive			1.75	0.90–3.40	0.094
CDD rs2072671					
Dominant					
AA	36 (40.4)	13 (31.7)	Referent		
AC/CC	53 (59.6)	28 (68.3)	1.72	0.74–3.97	0.201
Additive			1.55	0.80–2.99	0.190
CES2 rs2241409					
Dominant					
CC	52 (58.4)	26 (63.4)	Referent		
CT	37 (41.6)	15 (36.6)	0.71	0.31–1.60	0.403
Additive			0.78	0.40–1.52	0.456
CES2 rs11568314					
Codominant					
AA	78 (87.6)	37 (90.2)	Referent		
AT	11 (12.4)	4 (9.8)	0.74	0.20–2.71	0.648
CES2 rs11568311					
Codominant					
GG	76 (85.4)	36 (87.8)	Referent		
GA	13 (14.6)	5 (12.2)	0.81	0.25–2.60	0.715
CES2 rs11075646					
Dominant					
CC	69 (77.5)	29 (70.7)	Referent		
CG/GG	20 (22.5)	12 (29.3)	1.43	0.59–3.47	0.434
Additive			1.32	0.62–2.85	0.475
TP rs470119					
Codominant					
GG	38 (43.2)	19 (46.3)	Referent		
GA/AA	50 (56.8)	22 (53.7)	0.91	0.41–2.00	0.811
Additive			0.94	0.52–1.70	0.846
TP rs131804					
Codominant					
AA	32 (36.0)	14 (34.1)	Referent		
AG/GG	57 (64.0)	27 (65.9)	1.09	0.48–2.46	0.842
Additive			1.03	0.57–1.85	0.927
TP rs11479					
Codominant					
CC	75 (86.2)	37 (90.2)	Referent		
CT	12 (13.8)	4 (9.8)	0.76	0.21–2.68	0.664
TS 3′-UTR					
Dominant					
6bp/6bp	34 (38.2)	22 (53.7)	Referent		
6bp/del	55 (61.8)	19 (46.3)	0.55	0.25–1.21	0.138
Additive			0.67	0.37–1.21	0.172
TS 5′-UTR					
Dominant					
2R2R/2R3RC/3RC3RC	54 (62.1)	26 (63.4)	Referent		
2R3RG/3RC3RG/3RG3RG	33 (37.9)	15 (36.6)	1.10	0.49–2.49	0.821
Additive			1.02	0.53–1.93	0.960

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

Table 3. Genotype distribution and logistic regression analyses assessing associations of rs3215400 and rs603412 with grade 3 HFS

Genotype	*n* (%)		OR	95% CI	*P*
	Grade 0–2 HFS	Grade 3 HFS			
CDD rs3215400 943insC					
Dominant					
– –	23 (25.8)	19 (46.3)	Referent		
–*C*/*CC*	66 (74.2)	22 (53.7)	0.37	0.16–0.86	0.020
Additive			0.51	0.27–0.95	0.028
CDD rs603412 −205C>G					
Dominant					
CC	28 (31.8)	12 (29.3)	Referent		
CG/*GG*	60 (68.2)	29 (70.7)	1.19	0.50–2.80	0.693
Additive			1.41	0.77–2.60	0.261

Discussion

In the present study, we used a candidate pathway approach that analyzes polymorphisms across the genes responsible for metabolizing capecitabine and found an association between rs3215400 polymorphism in the *CDD* gene and grade 3 HFS.

The *CDD* gene encodes an enzyme involved in the pyrimidine salvage pathway and catalyzes irreversibly the hydrolytic deamination of cytidine and deoxycytidine to their corresponding uridine derivatives (10). In addition, *CDD* plays an essential role in the metabolism of a number of antitumor cytosine nucleoside analogues, leading to their pharmacologic activation to 5-FU.

To date, several studies have addressed the relationship between polymorphisms in the *CDD* gene and sensitivity to cytosine nucleoside analogues or related toxicities. The G208A polymorphism has been reported to be associated with 1β-D-arabinofuranosylcytosine sensitivity (19) and gemcitabine-related toxicities in the Japanese population (20), but it was not included in our study because it is almost monomorphic in the Caucasian population (12, 13). The 79A>C variant (rs2072671), which we did include in our study, has been reported to be related to gemcitabine sensitivity, but there is no clear evidence of a functional role or association with toxicities (19). Fitzgerald and colleagues (10) reported a difference in *CDD* expression considering haplotypes containing rs602950 and rs532545, but because this haplotype is very rare (≥1%) in our series of patients and in cell lines, it cannot account for the differences in expression observed. To our knowledge, only Ribelles and colleagues (9) have analyzed the association between the rs3215400 *CDD* polymorphism and HFS, but not significant correlation was found. Because of the limited sample size in the study of Ribelles and colleagues, their negative result could be a consequence of a lack of statistical power. Although the present study has been conducted using a limited number of patients as well, the further functional study conducted in cell lines

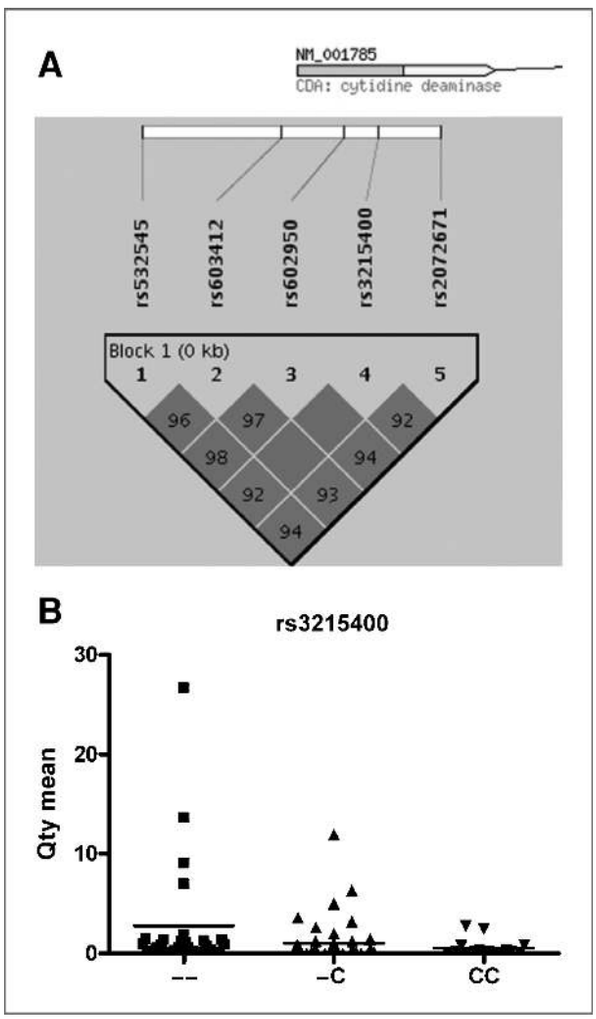

Figure 1. A, LD among the 5 variants studied at the *CDD* gene. Pairwise LD measures (*D'*) calculated with the software package Haploview (version 4.1) are shown. B, effect of the rs3215400 CDD polymorphism on gene expression in Coriell lymphoblastoid cell lines.

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

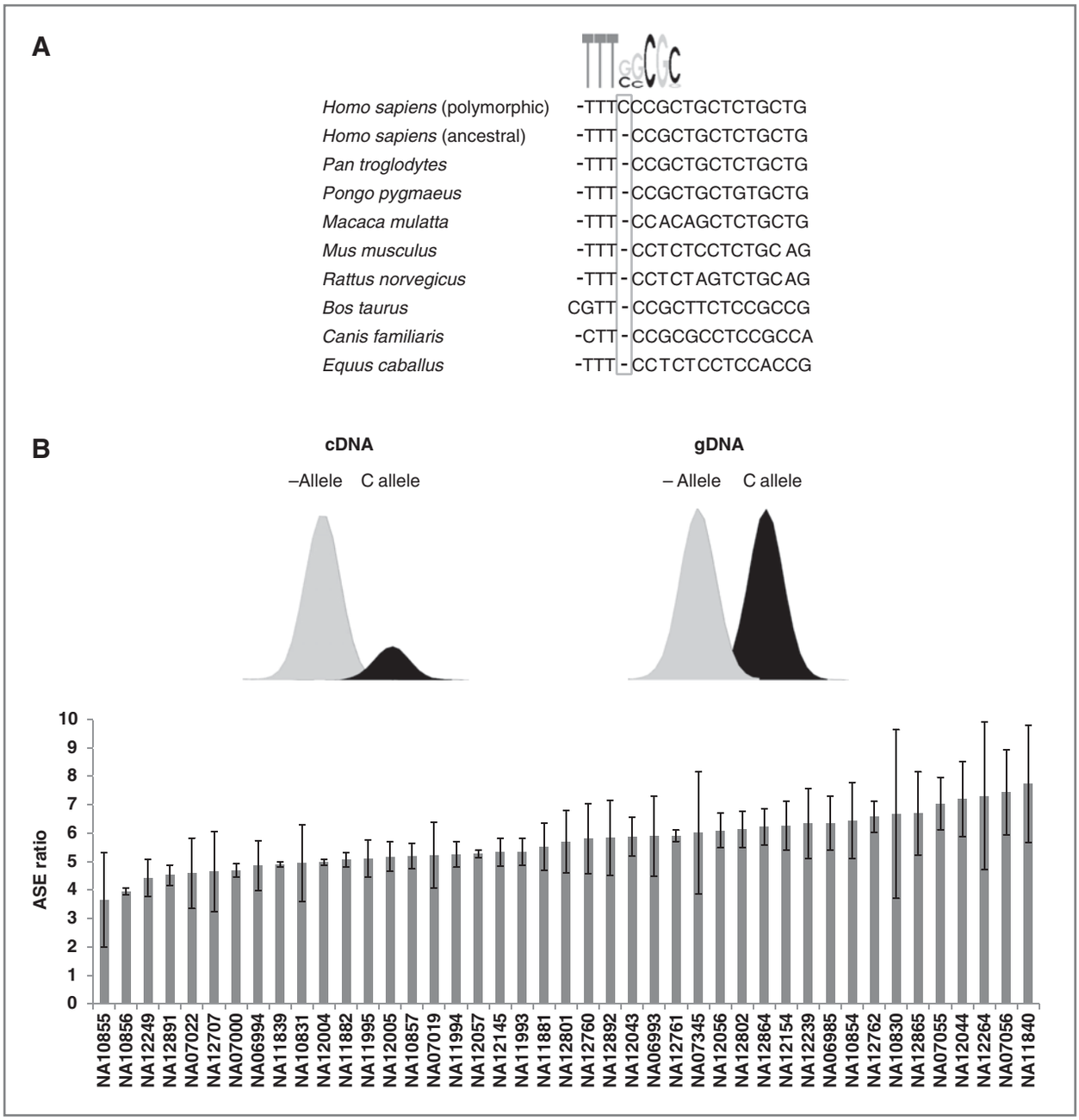

Figure 2. A, transcription factor binding site prediction: the rs3215400 *del* allele abrogates the E2F binding site. B, ASE analysis by SNaPshot in 43 Hapmap cell lines heterozygous for rs3215400. Top, an example of the peaks obtained for the deleted allele (gray) and for the C allele (black) for cDNA (left) and for genomic DNA (gDNA; right). The ASE (bottom) ratio was calculated by normalizing the ratio between the peak areas of the 2 alleles in cDNA for the same ratio in the genomic DNA.

supports our finding showing that the association observed in patients probably reflects the effect of this regulatory variant on *CDD* expression. Of the 5 common variants we analyzed at the *CDD* locus, rs3215400 found to be the most strongly associated with HFS. We hypothesized, based on *in silico* analysis, that the presence of a C allele at position 943 of the *CDD* gene is critical for transcriptional suppression of the *CDD* gene resulting in reduced CDD production and that such suppression is mediated through E2F binding. Such a functional explanation is in agreement with our observation that the deleted allele is associated with an increase in global *CDD* gene expression. We also found a consistent degree of ASE, with the deleted allele expressing 3- to 7-fold higher mRNA levels than the

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-1741

inserted allele, a result consistent with the global gene expression analysis. Because the functional experiments were done in lymphoblastoid cell lines show notable differences in the transcriptional activities of the deleted and C alleles, we infer that this could also happen in patients *in vivo* and therefore that the rs3215400 variant could be the causal variant.

On the basis of our findings, we propose a model in which the absence of an E2F site within the *CDD* promoter enhances CDD transcription. This could also take place in normal tissues. In particular, cell cytotoxicity could be accentuated by the elevated proliferation rate observed in the skin of the palm and sole, rendering them more sensitive to the cytotoxic effects of the 5-FU (21). This model may explain the capecitabine-related HFS variability found in treated patients.

Although our finding requires replication through more extensive and independent series of patients, our results provide evidence that rs3215400 in the *CDD* gene is a risk factor for HFS. An independent series of patients treated with capecitabine enrolled in a clinical trial (Standard Versus Continuous Capecitabine in Advanced Breast Cancer, NCT00418028) is being collected for validation.

This pharmacogenetic study provides new insight into the clinical toxicity associated with capecitabine treatment.

Disclosure of Potential Conflicts of Interest

M. Martin has received speaker's honoraria from Roche.

Acknowledgments

We thank Ariel Casanova and Paola Martinelli from the Epithelial Carcinogenesis Group and Barbara Rivera from the Human Genetics Group (Spanish National Cancer Research Centre) for the help in the ASE assays and Roger Milne for the statistical support.

Grant Support

The work was supported by the Foundation Genoma España and in part by the Spanish National Network of Cancer Centers (RTICC, RD06/0020/0021).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 1, 2010; revised October 21, 2010; accepted December 10, 2010; published OnlineFirst February 16, 2011.

References

1. McKendrick J, Coutsouvelis J. Capecitabine: effective oral fluoropyrimidine chemotherapy. Expert Opin Pharmacother 2005;6:1231–9.
2. Walko CM, Lindley C. Capecitabine: a review. Clin Ther 2005;27:23–44.
3. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 1998;34:1274–81.
4. Webster-Gandy JD, How C, Harrold K. Palmar-plantar erythrodysesthesia (PPE): a literature review with commentary on experience in a cancer centre. Eur J Oncol Nurs 2007;11:238–46.
5. Nagore E, Insa A, Sanmartin O. Antineoplastic therapy-induced palmar plantar erythrodysesthesia ("hand-foot") syndrome. Incidence, recognition and management. Am J Clin Dermatol 2000;1:225–34.
6. Ulrich CM, Bigler J, Bostick R, Fosdick L, Potter JD. Thymidylate synthase promoter polymorphism, interaction with folate intake, and risk of colorectal adenomas. Cancer Res 2002;62:3361–4.
7. van Kuilenburg AB. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. Eur J Cancer 2004;40:939–50.
8. Largillier R, Etienne-Grimaldi MC, Formento JL, Ciccolini J, Nebbia JF, Ginot A, et al. Pharmacogenetics of capecitabine in advanced breast cancer patients. Clin Cancer Res 2006;12:5496–502.
9. Ribelles N, López-Siles J, Sánchez A, González E, Sánchez MJ, Carabantes F, et al. A carboxylesterase 2 gene polymorphism as predictor of capecitabine on response and time to progression. Curr Drug Metab 2008;9:336–43.
10. Fitzgerald SM, Goyal RK, Osborne WR, Roy JD, Wilson JW, Ferrell RE. Identification of functional single nucleotide polymorphism haplotypes in the cytidine deaminase promoter. Hum Genet 2006;119:276–83.
11. Sugiyama E, Kaniwa N, Kim SR, Kikura-Hanajiri R, Hasegawa R, Maekawa K, et al. Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. J Clin Oncol 2007;25:32–42.
12. Fukunaga AK, Marsh S, Murry DJ, Hurley TD, McLeod HL. Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. Pharmacogenomics J 2004;4:307–14.
13. Gilbert JA, Salavaggione OE, Ji Y, Pelleymounter LL, Eckloff BW, Wieben ED, et al. Gemcitabine pharmacogenomics: cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. Clin Cancer Res 2006;12:1794–803.
14. Salgado J, Zabalegui N, Gil C, Monreal I, Rodriguez J, Garcia-Foncillas J. Polymorphisms in the thymidylate synthase and dihydropyrimidine dehydrogenase genes predict response and toxicity to capecitabine-raltitrexed in colorectal cancer. Oncol Rep 2007;17:325–8.
15. Wei X, McLeod HL, McMurrough J, Gonzalez FJ, Fernandez-Salguero P. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. J Clin Invest 1996;98:610–5.
16. van Kuilenburg AB, Muller EW, Haasjes J, Meinsma R, Zoetekouw L, Waterham HR, et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14+1G>A mutation causing DPD deficiency. Clin Cancer Res 2001;7:1149–53.
17. Mandola MV, Stoehlmacher J, Muller-Weeks S, Cesarone G, Yu MC, Lenz HJ, et al. A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res 2003;63:2898–904.
18. Bajetta E, Procopio G, Celio L, Gattinoni L, Della Torre S, Mariani L, et al. Safety and efficacy of two different doses of capecitabine in the treatment of advanced breast cancer in older women. J Clin Oncol 2005;23:2155–61.
19. Yue L, Saikawa Y, Ota K, Tanaka M, Nishimura R, Uehara T, et al. A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. Pharmacogenetics 2003;13:29–38.
20. Yonemori K, Ueno H, Okusaka T, Yamamoto N, Ikeda M, Saijo N, et al. Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. Clin Cancer Res 2005;11:2620–4.
21. Milano G, Etienne-Grimaldi MC, Mari M, Lassalle S, Formento JL, Francoual M, et al. Candidate mechanisms for capecitabine-related hand-foot syndrome. Br J Clin Pharmacol 2008;66:88–95.