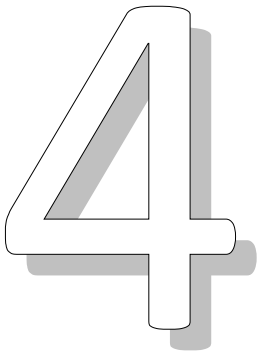
Epidermal growth factor receptor (EGFR) pathway polymorphisms as predictive markers of cetuximab toxicity in locally advanced head and neck squamous cell carcinoma (HNSCC) in a Spanish population



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Artículo 4: "Epidermal growth factor receptor (EGFR) pathway polymorphisms as predictive markers of cetuximab toxicity in locally advanced head and neck squamous cell carcinoma (HNSCC) in a Spanish population"

El tratamiento del CECC tiene un abordaje multidisciplinario incluyendo cirugía, radioterapia, quimioterapia basada en platino y el uso de fármacos frente a nuevas dianas terapéuticas, como es el caso del cetuximab, anticuerpo monoclonal IgG1 contra EGFR. Este fármaco desencadena sus efectos antitumorales a través de tres mecanismos diferentes: inhibición competitiva del ligando debido a su unión al dominio III extracelular del receptor, disminución del receptor en la membrana a través de endocitosis y degradación en el lisosoma; y, por último, la inducción de la citotoxicidad celular dependiente de anticuerpo (ADCC) a través de la interacción de la fracción constante del cetuximab con el receptor gamma (FcgR) portado por las células inmunes.

Aunque el uso de cetuximab está aprobado para el tratamiento de CECC localmente avanzado, el beneficio clínico y una baja toxicidad está restringido a un subgrupo de pacientes. La relación entre la presencia de toxicidad ante el tratamiento con cetuximab y una mejor respuesta ha sido ampliamente descrita. Para definir el subgrupo de pacientes que pueden beneficiarse de este tratamiento, se planteó un estudio de asociación entre los polimorfismos en la vía de señalización de EGFR y la toxicidad en pacientes con CECC.

Se estudiaron 110 pacientes con CECC localmente avanzado. La mayoría recibió cetuximab en concomitancia con quimioterapia o radioterapia administrada en una dosis inicial de 400mg/m^2 seguido por dosis semanales de 250mg/m^2 hasta la progresión de la enfermedad o toxicidad severa. Los datos de toxicidad fueron aportados en el cuestionario de recogida de datos según los criterios del NCI-CTCAE versión 3.0. Para realizar el análisis de los polimorfismos se extrajo el DNA de un tubo de sangre periférica estudiando los siguientes SNPs con sondas Taqman®: *EGFR* rs2227983, rs28384375 y rs17336639; *KRAS* rs61764370, *FCGR2A* rs180127 y *FCGR3A* rs396991, mientras que por PCR y posterior digestión con enzimas de restricción se analizó el SNP en *CCND1* rs603965.

La toxicidad específica tras el tratamiento con cetuximab se presentó como *rash* acneiforme en un 55.5%, sequedad de piel con un 45.5% y prurito en un 20.9%. Esta toxicidad fue independiente de los ciclos de cetuximab recibidos ($p>0.05$). Los pacientes de este estudio solo presentaban el alelo común en los SNPs rs28384375 y rs17336639 de *EGFR* y fueron descartados del estudio.

El análisis estadístico demostró una asociación estadísticamente significativa entre el alelo G del SNP rs61764370 de *KRAS* y una menor aparición de sequedad de piel o toxicidad global (considerando la presencia de cualquier tipo de toxicidad). Este hecho puede ser debido a la presencia de este SNP en una zona de unión de miRNA de la familia let-7 en el extremo 3´-UTR del gen *KRAS*, asociado a una menor inhibición y mayor expresión del gen. La presencia de menor toxicidad puede relacionarse con peor respuesta al tratamiento y progresión tumoral, por un aumento de la expresión del oncogén *KRAS*.

De modo similar, el polimorfismo rs2227983 de *EGFR* demostró una asociación entre el alelo A y un menor riesgo a desarrollar prurito. Una posible explicación podría ser que el SNP produce un cambio de lisina a arginina en la región extracelular, produciendo un cambio estructural en el receptor, disminuyendo la interacción del cetuximab, asociándose a una menor efectividad de la diana terapéutica y a menor toxicidad.

Aunque no significativo (p=0.051), se observó una tendencia entre el genotipo TT del SNP *FCGR2A* y un menor riesgo a desarrollar sequedad de piel. Esto podría ser debido a que el polimorfismo causa un cambio de histidina a arginina, teniendo el alelo T, codificante para histidina, una menor afinidad por el cetuximab, causando menor toxicidad debido a una menor citotoxicidad antitumoral mediada por anticuerpos (ADCC). El resto de polimorfismos no mostraron asociación con ningún tipo de toxicidad producida por el cetuximab.

Este estudio aporta una evidencia preliminar de biomarcadores en genes implicados en la vía de señalización de EGFR, prediciendo la toxicidad y respuesta ante el tratamiento de CECC con cetuximab, lo que permitiría identificar aquellos pacientes que se beneficiarían de este tratamiento o deberían ser alternados a otras opciones terapéuticas.

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Epidermal growth factor receptor (EGFR) pathway polymorphisms as predictive markers of cetuximab toxicity in locally advanced head and neck squamous cell carcinoma (HNSCC) in a Spanish population

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ABSTRACT

Objectives: To examine the relationship between polymorphisms of the epidermal growth factor receptor (EGFR) pathway and toxicity in head and neck squamous cell carcinoma (HNSCC) patients treated with cetuximab.

Material and methods: Multicenter, retrospective, observational pilot study which included 110 patients with histologically-confirmed human papillomavirus (HPV) negative HNSCC in locally advanced stages (III-IVA-B) and who were treated with chemotherapy and radiotherapy plus cetuximab between 2003 and 2013. Genetic analyses for single nucleotide polymorphisms (SNP) in genes EGFR, CCDN1, FCGR2A, FCGR3A and KRAS-LCS6 were performed though available allelic discrimination assay and/or polymerase chain reaction-restriction fragment length polymorphism methods.

Results: Acneiform rash was observed in 55.5% of patients, dry skin in 45.5% and pruritus in 20.9%. A significant association with dry skin and global cetuximab-related toxicity was observed for the KRAS-LCS6 (rs61764370) variant ($p < 0.05$); carriers of the G allele (genotypes TG + GG) in the dominant model were observed to have a decreased susceptibility of developing dry skin (OR = 0.287 [95%CI = 0.119–0.695]). Carriers of the A (GA + AA) allele for EGFR (rs2227983) showed a decreased risk of suffering from pruritus (OR = 0.345 [0.124–0.958]). Similarly, KRAS (rs1801274) was related with lower global cetuximab-related toxicity (OR = 0.266 [0.114–0.622]).

Conclusion: This pilot study provides preliminary evidence supporting genetic variation of EGFR (rs2227983), KRAS (rs61764370) and FCGR2A (rs180127) as useful biomarkers for predicting reduced skin toxicity in HNSCC patients treated with a cetuximab-based therapy. Alternative therapeutic options should be explored for these patients.

Introduction

Head and Neck Carcinoma (HNC) includes a large number of tumors located in different anatomical regions of the upper aerodigestive tract. More than 90% of HNC tumors have a squamous cell histology [1] and are classified as head and neck squamous cell carcinoma (HNSCC), the sixth most common cancer worldwide [2]. Tobacco use and alcohol consumption are the most relevant

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etiological factors showing additive effect [3,4]. Nevertheless, in the last decades viral infection by the Epstein-Barr virus (EBV) or human papillomavirus (HPV) have been suggested as a cause of nasopharyngeal and oropharyngeal cancer, respectively. These EBV or HPV positive tumors appear to be clinically and molecularly different from negative virus carcinomas [5]. In addition, genetic variation in the germinal cell line has been found to modify the risk of disease and patient survival [3,6]. Despite their common squamous origin, the prognosis of these tumors primarily depends on their size and the presence of cervical lymph node and/or distant metastasis. Treatment options for HNSCC includes surgery, radiotherapy, platinum-based chemotherapy and targeted therapeutic agents [7]. However, these patients usually achieve an advanced-staged diagnosis that compromises first line response rates [8].

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor that plays a fundamental role in signal transduction pathways involved in DNA repair, tumor cell survival (PI3K-PTEN-AKT pathway), cell proliferation (RAS-RAF-MAPK pathway) and metastasis [9]. More than 95% of HNSCC patients have EGFR overexpression and it has been associated with a decreased response to therapy, reduced disease-free and overall survival (OS) [5]. Due to its prevalence and crucial role in pathogenesis, targeting EGFR has become a rational approach for HNSCC treatment.

Cetuximab, a chimeric mouse/human IgG1-type monoclonal antibody (MAb), is an anti-EGFR therapy approved for the treatment of locally advanced HNSCC [10,11]. Cetuximab in combination with radiotherapy or platinum-containing chemotherapy regimens has already shown significant improvement of treatment outcomes in metastatic and relapsed disease [7,10–15].

Cetuximab can cause antitumor effects through three different mechanisms: firstly, it specifically binds to the extracellular domain III of the EGFR as a competitive inhibitor of the natural EGF ligands and downstream pathway activation [16]. Secondly, cetuximab decreases the number of EGFRs in the tumor cell membrane though EGFR-cetuximab complex endocytosis and destruction by lysosomes. Thirdly, cetuximab can induce antibody-dependent cell-mediated cytotoxicity (ADCC) [17] through the interaction of the Fc region of the monoclonal antibody with the Fc gamma receptor (FcgR) carried by macrophages and natural killer cells [18,19].

Clearly, clinical benefit and low toxicity with EGFR-targeting antibodies seems to be restricted to a particular subgroup of HNSCC patients [20]. Although critically required for managing the high cost of this type of therapy and their anticipated integration in other clinical regimens, no validated predictive factors are currently available to improve treatment decision making [20]. Thus, it appears necessary to better define the subpopulation of patients who truly benefits from cetuximab treatment and its toxicity. Single nucleotide polymorphisms (SNP) may affect pharmacodynamics of anti-EGFR therapies introducing inter-patient variability at the level of the EGFR target itself, the downstream cascade, as well as at the ADCC. It has been reported that two SNPs located in the coding region of the FcgR have been associated with differences in the response and toxicity to cetuximab: a histidine (H)/arginine(R) polymorphism at position 131 of FCGR2A (rs1801274) and a valine(V)/phenylalanine(F) polymorphism at position 158 in FCGR3A (rs396991) [18,19,21,22]. At least three functional EGFR variants have been associated with EGFR regulation: rs2227983 [23], rs28384375 and rs17336639 [24,25] coding for amino acids located at the extracellular domain.

Moreover, some downstream effectors of EGFR signaling such as cyclin-D1 gene (CCDN1) may also plays a role in modulating cetuximab activity, given that CCND1 A870G (rs603965) polymorphism is positively correlated with HNSCC patient survival [26]. Finally, microRNA (miRNAs) – small non-coding RNAs able to suppress translation through their binding to the gene 3′-untanslated region (UTR) or inducing mRNA degradation – [27], can regulate KRAS activity, i.e. let-7 miRNA. A polymorphism in LCS6 (rs617764370) modifies let-7 binding affinity and it was associated with increased KRAS expression in an *in vitro* model, reducing survival in oral cancer and improving patient response to cetuximab [28].

Several studies have also found a relationship between skin toxicity, the most relevant cetuximab-related secondary effect [10,29], and a better response [30–32].

Therefore, the main objective of this study was to examine the possible associations between polymorphisms at genes coding for EGFR, CCDN1, FCGR2A, FCGR3A and KRAS-LCS6 and toxicity in HNSCC patients treated with cetuximab.

Materials and methods

Patients and treatment

A total of 110 patients with histologically-confirmed HPV-negative HNSCC were enrolled in a multicenter retrospective observational pilot study coordinated by the Medical Oncology Service of the University Hospital of Salamanca, Spain. All patients included in the study were diagnosed with locally advanced stages (III-IVA-B) and treated with chemotherapy and radiotherapy plus cetuximab between 2003 and 2013. The study was carried out after ethics committee approval and collection of informed consent from each patient. Patient tumor characteristics (location and stage) and data related to treatment (radiotherapy, chemotherapy and EGFR targeted therapy) and specific toxicity were compiled in a case report form (CRF) questionnaire by a medical oncologist.

The inclusion criterion was patients with a confirmed oral cavity, larynx, hypopharynx or oropharynx HNSCC diagnosis and who were treated with cetuximab. Cetuximab alone (n = 2) or with chemotherapy (n = 6), radiotherapy (n = 21), or radiochemotherapy (n = 81) was administered at an initial dose of 400 mg/m^2 followed by subsequent weekly dose of 250 mg/m^2 until disease progression or severe toxicity. Toxicity was recorded according to National Cancer Institute Common Toxicity Criteria (NCI-CTCAE), version 3.0. Exclusion criteria were uncertain or debatable diagnosis, benign tumors, and HPV-positive HNSCC confirmed by either PCR or immunohistochemistry.

DNA isolation and polymorphisms genotyping

Samples were obtained by venipuncture of a peripheral vein. DNA was extracted from leukocytes by phenol-chloroform extraction. Genetic analysis was performed using TaqMan® Allelic Discrimination Assay (Applied Biosystems) for SNPs for which TaqMan® probes were designed (Table 1). In these cases, 40 ng/μl of each sample were added to 6.25 μl of Taqman® Universal PCR Master Mix and 12.5 μl of reaction was combined with specific forward and reverse primers, and allele-specific VIC (allele 1) and FAM (allele 2) labeled probes. The assay was performed in a 96 well plate and the detection was measured in the Applied Biosystems Step One Plus instrument where the thermal cycling and detection was carried out. Negative and positive controls were always added [33].

The CCDND1 A870G polymorphism (rs603965) was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The forward primer was: 5′-GTGAAGTT CATTTCCAATCCGC-3′ and the reverse: 5′-GGGACATCACCCTCACT TAC-3′. Digestion was made by the restriction enzyme *ScrFI*. The PCR products were run on 3% Syber-safe stained agarose gel and visualized under UV light [34].

Table 1
Polymorphisms analyzed by TaqMan® probes in HNSCC patients.

SNP	RefSNP	Location	Context sequence [VIC/FAM]
EGFR R521K	rs2227983	Chr.7: 55161562	GAGGGCTGCTGGGGCCCGGAGCCCA**[A/G]**GGACTGCGTCTCTTGCCGGAATGTC
EGFR V592A	rs28384375	Chr.7: 55233025	CACTACATTGACGGCCCCCACTGCG**[C/T]**CAAGACCTGCCCGGCAGGAGTCATG
EGFR P266R	rs17336639	Chr.7: 55154060	GCCACGTGCAAGGACACCTGCCCCC**[C/G]**ACTCATGCTCTACAACCCCACCACG
KRAS-LCS6	rs61764370	Chr.12:25207290	F:5′-GCCAGGCTGGTCTCGAA-3′ R:5′-CTGAATAAATGAGTTCTGCAAAACAGGTT-3′ CTCAAGTGAT**[T/G]**CACCAC
FCGR2A H131R	rs1801274	Chr.1: 161509955	AATGGAAAATCCCAGAAATTCTCCC**[A/G]**TTTGGATCCCACCTTCTCCATCCCA
FCGR3A V18F	rs396991	Chr.1: 161544752	TCTGAAGACACATTTTTACTCCCAA**[C/A]**AAGCCCCCTGCAGAAGTAGGAGCCG

Statistical analysis

Statistical analysis compared categorical parameters and polymorphism status by the Chi-square test. P-values were considered statistically significant when $p < 0.05$. Significant variables were included in the logistic regression analysis and size effects were indicated by odds ratio (OR) with their 95% confidence interval (95% CI). Dose and toxicity influence was analyzed by a Mann-Whitney *U* test due to the non-parametric distribution of the variables. Secondary endpoint was cetuximab-related toxicity. All these tests were conducted using SPSS software 21.0 version for Windows (SPSS Inc., Chicago).

Toxicity was graded between 0 = absence and grade 4 = severe, grouped as low (1–2) or high (3–4) grade, and classified as "present" or "absent". The term global toxicity was applied if the patients presented some grade of toxicity to the treatment.

Results

A total of 110 locally advanced HNSCC patients were analyzed. Most of the patients included in the study were men with a median age of diagnosis of 59.63 years old. Regarding tumor characteristics, the most common were laryngeal neoplasias (45.5%), followed by pharyngeal (41.8%) and oral cavity (12.7%); the majority of tumors were stage IV (70.0%), followed by stage III (30.0%) (Table 2). As for the therapy received in combination with cetuximab, 6 (5.5%) patients were treated with chemotherapy, 21 (19.1%) with radiotherapy and 81 (73.6%) with radiochemotherapy (Table 3). Statistical association was not found in the comparison between patients undergoing radiochemotherapy and cetuximab versus the remaining therapies ($p > .05$), thus indicating that the toxicity between the two groups was likely produced by the cetuximab treatment itself (Table 3).

Regarding the specific toxicity of cetuximab, 55.5% of HNSCC patients presented acneiform rash, 46.4% of them in low grade; while dry skin was present in 45.5% of cases and pruritus in 20.9% (Table 4). Despite a variation in the number of cetuximab cycles, mean 10.54 ± 15.04, no statistically-significant relationship was observed between accumulated doses and toxicity in the Mann-Whitney *U* test (dry skin $p = 0.116$, pruritus $p = 0.787$ and rash $p = 0.284$).

Table 2
Characteristics of HNSCC patients.

		N	%
Sex	Male	100	90.9
	Female	10	9.1
Location	Larynx	50	45.5
	Oropharynx	30	27.3
	Hypopharynx	16	14.5
	Oral cavity	14	12.7
Stage	III	33	30.0
	IVA	65	59.1
	IVB	12	10.9

The genotype distribution of EGFR rs2227983, rs28384375 and rs17336639, FCGR2A rs1801274, FCGR3A rs396991, KRAS-LCS6 rs61764370, and CCDN1 rs603965 polymorphisms are shown in Table 5. EGFR polymorphisms rs28384375 and rs17336639 had only the major allele variant in our sample (Table 5), though in the European population rs28384375 has been described as having a distribution of 84% CC, 14.2% CT and 1.2% TT, and for rs17336639: 98.9% CC and 1.1% CG. Thus they were not included in subsequent analyses. The remaining SNPs were analyzed according to the most common toxicity produced by monoclonal antibodies treatment: dry skin, pruritus and acneiform rash.

Statistical analyses using the Chi-square test showed significant association between dry skin and the KRAS-LCS6 (rs61764370) variant ($p < 0.05$). Moreover, global cetuximab-related toxicity was also associated with this polymorphism, and may be due to the association with dry skin toxicity (Table 6). These results showed that being a carrier of the G allele (genotypes TG + GG) of the KRAS-LCS6 rs61764370 polymorphism in the dominant model decreases the susceptibility to develop dry skin after cetuximab treatment ($p = 0.006$, OR = 0.287 [95% CI = 0.119–0.695]) (Table 7). Although not significant, a tendency in the recessive model of FCGR2A rs1801274 where the TT genotype was close to being associated with a decreased risk of dry skin, $p = 0.051$ OR = 0.380 (0.144–1.003), was observed (Table 7). Secondly, the EGFR rs2227983 polymorphism showed an association with pruritus toxicity. Carriers of GA + AA genotypes were found to have a decreased risk of suffering from pruritus: $p = 0.041$, OR = 0.345 (0.124–0.958) (Table 7). Regarding patients with global cetuximab-related toxicity, the KRAS (rs61764370) variant was less susceptible to global toxicity related to MAb treatment ($p = 0.002$, OR = 0.266 [95% CI = 0.114–0.622]) (Table 7).

Discussion

Cetuximab combined with radiotherapy or chemotherapy improves locoregional control and survival in HNC patients, but only a subset of all patients are able to benefit from anti-EGFR monoclonal antibodies [35]. Thus, the detection of predictive biomarkers and of beneficial patient profiles is crucial. Several studies have correlated clinical outcome and toxicity to IgG1 cetuximab treatment with polymorphisms in the EGFR pathway with conflicting results [18,19,21,22,26,28]. In this proof of concept study we evaluated the possibility of an association between cetuximab toxicity and polymorphism distribution in the EGFR pathway, looking for predictive biomarkers of toxicity.

Skin toxicity is a frequent side effect of EGFR targeting agents and it correlates with a better treatment efficacy [32]. It causes some cutaneous changes such as acneiform rash, dry skin and itching. Although these toxicities can negatively impact on the patient quality of life, the identification of new biomarkers may contribute

Table 3
Treatment and cetuximab-related toxicity.*

			Acneiform Rash		Dry skin		Pruritus		Global toxicity	
	N	%	N	%	N	%	N	%	N	%
Cetuximab alone	2	1.8	19	65.5	15	51.7	4	13.8	22	75.9
Cetuximab + Radiotherapy	21	19.1								
Cetuximab + Chemotherapy	6	5.5								
Cetuximab + Radiochemotherapy	81	73.6	42	51.9	35	43.2	19	23.5	51	63.0

* The data represents only patients who developed toxicity. A Chi-Square test was performed between patients undergoing radiochemotherapy plus cetuximab versus the remaining therapies and there were not statistical differences between both groups ($p > .05$) (data not shown).

Table 4
Toxicity caused by cetuximab therapy, clustered by low (1–2) and high (3–4) grade.

	Acneiform rash		Dry skin		Pruritus	
	N	***%***	***N***	***%***	***N***	***%***
Absence	49	44.5	60	54.5	87	79.1
G1-2	51	46.4	41	37.3	22	20.0
G3-4	10	9.1	9	8.2	1	0.9

Table 5
Distribution of polymorphism genotypes in this sample.

SNP	Genotype frequency		
EGFR rs2227983	GG	GA	AA
	60 (54.5%)	43 (39.1%)	7 (6.4%)
EGFR rs28384375	CC	CT	TT
	110 (100%)	0 (0%)	0 (0%)
EGFR rs17336639	CC	CG	GG
	110 (100%)	0 (0%)	0 (0%)
FCGR2A rs1801274	CC	CT	TT
	17 (15.5%)	68 (61.8%)	25 (22.7%)
FCGR3A rs396991	TT	TG	GG
	39 (35.5%)	55 (50.0%)	16 (14.5%)
KRAS-LCS6 rs61764370	TT	TG	GG
	75 (68.2%)	31 (28.2%)	4 (3.6%)
CCDN1 rs603965	AA	AG	GG
	29 (26.4%)	57 (51.8%)	24 (21.8%)

to being able to predict the patient who will develop toxicity and thus have a better response to treatment. EGFR is normally found in keratinocytes of the epidermis, follicular epithelium and sweat glands. This receptor has an important function in skin homeostasis and its inhibition drives to an abnormal proliferation and differentiation of the epithelium [36].

In this study no relationship between genotype distribution of EGFR rs28384375, rs17336639, FCGR2A rs1801274, FCGR3A rs39661 and CCDN1 rs603965 gene polymorphisms and cetuximab toxicity was observed for this patient population. However, an association between EGFR rs2227983 and pruritus development after cetuximab treatment was observed. The EGFR SNP rs2227983 G > A in exon 13 produces a change of arginine to lysine in the position 521 (R521K). Previous reports have noted that carriers of the A allele (AA or GA) were associated with a lower incidence of skin rash compared with the GG genotype in advanced HNC [32]. In this study AA + GA was associated with a lower risk of developing pruritus ($p = 0.041$; OR = 0.345 [0.124–0.958]). Although this relationship remains unclear, this SNP is located in the extracellular region, where the monoclonal antibody and the EGF ligand interact, so structural changes at codon 521 could provoke a modification of EGF interaction with the receptor. In conclusion, AA genotype could be related with decreased cetuximab binding, low effectiveness of the monoclonal antibody and less toxicity, also clearly related with a lower response [32].

In this study, KRAS rs617764370 SNP was observed to be associated with lower dry skin and global cetuximab-related toxicity. MicroRNA SNPs are arising as relevant molecular markers in personalized medicine. The KRAS-LCS6 variant has a functional impact on let-7 miRNA joining to 3′-UTR of KRAS gene [28], causing less inhibition and an increased KRAS expression [28]. KRAS, a downstream EGFR effector, is involved in cell proliferation and maintain skin homeostasis [36]. KRAS rs61764370 has been also associated with reduced OS in oral cancer [28]. Our results show a lower risk of developing skin or global toxicity in variant carriers probably due to a higher KRAS activity. If early skin toxicity predicts better outcome and response after cetuximab treatment [30,32], these results indicate that lower toxicity is related with worse response and tumor progression after cetuximab treatment, associated to higher KRAS expression. Moreover, EGFR inhibition has been previously associated with higher grade of skin toxicity, due the lower activity of downstream signal, inducing inflammatory response [36]. As KRAS is an important effector on the pathway that maintains skin homeostasis, the increase of KRAS expression due to variant rs61764370 could activate important transcription factors to keep skin homeostasis and reducing skin toxicity [36].

Table 6
P-values of different polymorphism selected comparing grade of toxicity (as shown in Table 3) and the presence or absence of the event.

		Rash acneiforme		Dry skin		Pruritus		Global toxicity
Polymorphism	Gene	Grade	Yes/no	Grade	Yes/no	Grade	Yes/no	Yes/no
rs2227983	EGFR	0.808	0.863	0.917	1.000	0.172	0.079	0.853
rs1801274	FCGR2A	0.474	0.410	0.101	0.051	0.620	0.446	0.138
rs396991	FCGR3A	0.285	0.274	0.497	0.185	0.646	0.694	0.292
rs61764370	KRAS	0.389	0.135	**0.039**	**0.013**	0.657	0.411	**0.003**
rs603965	CCDN1	0.437	0.512	0.974	0.819	0.357	0.147	0.688

Statistically significant results in bold.

Table 7
Distribution of genotypes associated to skin/global toxicity.

SNP	Genotype	Patients with toxicity	Patients without toxicity	p-Value	OR (95% IC)
KRAS rs61764370 in association with dry skin	TT	41 (82.0%)	34 (56.7%)	/	1.00
	TG	8 (16.0%)	23 (38.3%)	**0.008**	**0.288 (0.114–0.727)**
	GG	1 (2.0%)	3 (5.0%)	0.275	0.276 (0.027–2.780)
	TT + TG	49 (98.0%)	57 (95.0%)	/	1.00
	GG	1 (2.0%)	3 (5.0%)	0.419	0.388 (0.039–3.849)
	TT	41 (82.0%)	34 (56.7%)	/	1.00
	TG + GG	9 (18.0%)	26 (43.3%)	**0.006**	**0.287 (0.119–0.695)**
FCGR2A rs1801274 in association with dry skin	CC	6 (12.0%)	11 (18.3%)	/	1.00
	CT	37 (74.0%)	31 (51.7%)	0.164	2.188 (0.726–6.595)
	TT	7 (14.0%)	18 (30.0%)	0.616	0.713 (0.190–2.678)
	CC + CT	43 (86.0%)	42 (70.0%)	/	1.00
	TT	7 (14.0%)	18 (30.0%)	**0.051**	**0.380 (0.144–1.003)**
	CC	6 (12.0%)	11 (18.3%)	/	1.00
	CT + TT	44 (88.0%)	49 (81.7%)	0.363	1.646 (0.562–4.823)
EGFR rs2227983 in association with pruritus toxicity	GG	17 (73.9%)	43 (49.4%)	/	1.00
	GA	6 (26.1%)	37 (42.6%)	0.090	0.410 (0.147–1.148)
	AA	0 (0.0%)	7 (8.0%)	0.832	0.999 (0.000-)
	GG + GA	23 (100.0%)	80 (92.0%)	/	1.00
	AA	0 (0.0%)	7 (8.0%)	0.999	0.000 (0.000-)
	GG	17 (73.9%)	43 (49.4%)	/	1.00
	GA + AA	6 (26.1%)	44 (50.6%)	**0.041**	**0.345 (0.124–0.958)**
KRAS rs61764370 in association with global toxicity	TT	60 (77.9%)	24 (50.0%)	/	1.00
	TG	16 (20.8%)	21 (43.8%)	**0.007**	**0.296 (0.123–0.715)**
	GG	1 (1.3%)	3 (6.2%)	0.058	0.105 (0.010–1.076)
	TT + TG	76 (98.7%)	45 (93.8%)	/	1.00
	GG	1 (1.3%)	3 (6.2%)	0.115	0.115 (0.016–1.569)
	TT	60 (77.9%)	24 (50.0%)	/	1.00
	TG + GG	17 (22.1%)	24 (50.0%)	**0.002**	0.266 (0.114–0.622)

Statistically significant results in bold.

ADCC is one of the secondary pathways through cetuximab exerts its antitumor effect. Recently, two FCGR SNPs have been identified that affect the binding strength to IgG1, varying ADCC function and affecting clinical tumor response [22]. In this study no association was observed between FCGR3A rs39661 and cetuximab toxicity. However, rs180127 (FCGR2A H131R) was close to being associated with skin toxicity (p = 0.051). These results show that genotypes with the allele variant T (TT), which encodes for histidine, could emerge as a possible predictor of reduced cetuximab skin toxicity (OR = 0.380 [0.144–1.003]). A putative explanation could be that FCGR2A expressing macrophages would play an important role in restoring tumor immune surveillance as predicted in preclinical models [21]. It is known that FCGR2A-131 H/H genotype has higher affinity to human IgG2 than131R allele [19] and it is associated with longer progression-free survival in cetuximab monotherapy. Cetuximab is a IgG1 antibody, and 131H has been related with low affinity binding to murine IgG1 [22]. Thus, in those situations IgG1 binds more strongly to FCGR2A 131R, and ADCC antitumor response can be less effective. As a direct relationship between skin toxicity and better MAb response has been documented [30–32], these results suggest that lower affinity to IgG1 recognition in patients with T allele could result in less toxicity to MAb treatment probably due to lowest antitumor cytotoxicity.

Lastly, cyclin-D1 gene (CCDN1), a downstream effector of EGFR is also involved in cetuximab activity. No association was observed between the CCND1 870A > G (rs603965) polymorphism and EGFR MoAb toxicity, an association that has been documented elsewhere with survival in patients with colorectal cancer treated with cetuximab [37].

Conclusions

This pilot study provides preliminary evidence supporting EGFR rs2227983, KRAS rs61764370 and FCGR2A rs180127 as useful biomarkers for predicting reduced skin toxicity in HNSCC patients receiving cetuximab-based therapy. This could indicate that patients with these genetic variants could have less toxicity and a poor prognosis, being better scheduled in another therapeutic alternative. Although these polymorphisms are checked in HNSCC cetuximab-related toxicity in this study for the first time, they should be interpreted carefully. The statistical power of this study is limited due to the moderate number of analyzed patients. Studies in larger groups should be performed and would be necessary to confirm these results and validate our findings.

Conflict of interest statement

All authors declare no conflict of interest in relation to this manuscript.

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