Genetic variations in *EGF* and *EGFR* and glioblastoma outcome

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Few prognostic factors have been associated with glioblastoma survival. We analyzed a complete tagging of the epidermal growth factor (EGF) and EGF receptor (EGFR) gene polymorphisms as potential prognostic factors. Thirty tagging single-nucleotide polymorphisms (SNPs) in *EGF* and 89 tagging SNPs in *EGFR* were analyzed for association with survival in 176 glioblastoma cases. Validation analyses were performed for 4 SNPs in a set of 638 glioblastoma patients recruited at The University of Texas M. D. Anderson Cancer Center (MDACC). Three hundred and seventy-four glioblastoma patients aged 50 years or older at diagnosis were subanalyzed to enrich for de novo arising glioblastoma. We found 7 SNPs in haplotype 4 in *EGF* that were associated with prognosis in glioblastoma patients. In *EGFR*, 4 of 89 SNPs were significantly associated with prognosis but judged as false positives. Four of the significantly associated *EGF* polymorphisms in haplotype block 4 were validated in a set from MDACC; however, none of the associations were clearly replicated. rs379644 had a hazard ratio (HR) of 1.19 (0.94–1.51) in the whole population with 18.6 months survival in the risk genotype compared with 24.5 in the reference category. As the median age differed slightly between the 2 study sets, the MDACC cases aged 50 or older at diagnosis were analyzed separately (rs379644, HR 1.32 [0.99–1.78]), which is marginally significant and partially validates our findings. This study is, to our knowledge, the first to perform a comprehensive tagging of the *EGF* and *EGFR* genes, and the data give some support that EGF polymorphisms might be associated with poor prognosis. Further confirmation in independent data sets of prospective studies is necessary to establish EGF as prognostic risk factor.

Keywords: EGF, EGFR, glioblastoma, outcome, polymorphism.

Glioblastoma is the most common and aggressive type of glioma. It is associated with poor prognosis; however, few prognostic factors have been identified apart from age, Karnofsky performance status, and MGMT hypermethylation status. Low penetrance genes (single-nucleotide polymorphisms [SNPs]) in epidermal growth factor (*EGF*)[1,2] have been reported to be prognostic factors, and previous studies have investigated a smaller set of polymorphism in immune function genes such as interleukin 4 (*IL4*),[3] HTERT,[4,5] and glutathione metabolism.[3,6] Few previous studies have investigated genes by complete gene tagging. An association with poor survival of glioblastoma patients and a functional polymorphism, the 61 A/G located in the *EGF* promoter, was observed in a small study including 42 patients with glioblastoma,[1] but 2 other studies have failed to replicate the initial observation in 197 and 200 glioblastoma cases, respectively.[2,7] In a normal tissue, the ligand EGF binds to the EGF receptor (*EGFR*), inducing a dimerization of one or several members of the *EGF* receptor family (*ErbB* 1–4), activating several tyrosine kinases and other downstream signal molecules

promoting transcription in the cell nucleus. The *EGFR* gene plays a key role in the glioma progression pathway and is amplified in approximately 30%–50% of malignant gliomas. Glioblastoma with *EGFR* amplifications often contain a genetic mutation of *EGFR* (*EGFR*vIII); this mutation alters the receptor domain and renders the receptor to be constitutively active by phosphorylation.[8] EGFR overexpression in tumor samples of low-grade glioma[9] and anaplastic astrocytoma[10] has been associated with poor prognosis. As the EGFR signaling pathway is a key feature in glioma progression, our aim was to perform comprehensive tagging and analyzes of germline genetic variants in *EGF* and *EGFR* to evaluate if these genes play a role in glioblastoma prognosis.

Material and Methods

Study Subjects

The initial study set was based on 176 cases from Sweden and Denmark diagnosed with glioblastoma (International Classification of Diseases [ICD] code 94403) between the years 2000 and 2004, as part of the international Interphone study.[11] Association studies have previously been done on this material, and the cases' ascertainment details are described in previous publications.[5,12] Eligible cases were invited by letter or personally to take part in the study. If no reply was received, a repeat letter was sent or the potential participant was contacted by telephone. Blood samples were collected from all cases, and a pathology review was conducted by a Swedish and Danish pathologist. The tumors were graded and classified according to the WHO 2007 criteria.[13] The age of the cases ranged between 20 and 69,

and there were 109 men and 67 women. The mean age at diagnosis was 54 and the median age at diagnosis was 56 years. For these cases, all medical records were retrieved and detailed clinical data were collected, including date of diagnosis, follow-up date(s), surgery, radiotherapy treatment, and treatment programs including chemotherapy. In 33 cases, only incomplete information on treatment was available; chemotherapy only in 23 cases, radiotherapy (1 case), and/or surgery in 9 cases. In 6 of these cases, we were not able to collect any treatment data at all (3%). All cases were followed from date of diagnosis until either date of death or date of last follow-up in the patient records. In 10 cases, follow-up was not available and, in an additional 11 cases, we lacked information on the date of diagnosis, leaving 155 cases for the final analysis (Table 1). The missing values were due to lack of available medical reports as the patients moved several times, which did not enable the collection of complete follow-up information.

Conformational analyses on significant SNPs in *EGF* were made using a data set of 638 glioblastoma cases recruited from 1990 to 2008, with follow-up until April 2009, from The University of Texas M. D. Anderson Cancer Center (MDACC). All cases were Caucasians, had a confirmed diagnosis of primary glioblastoma (ICD-9 codes 94403), and were at least 18 years of age at the time of diagnosis. The male/female ratio was 397/241, the mean age at diagnosis was 52.3 years, and median age at diagnosis was 54 years. In this data set, 586 cases were deceased and 52 cases were alive at the last follow-up. Cases were excluded if they had a prior diagnosis of cancer or if they had undergone prior treatment for their glioblastoma (radiotherapy, chemotherapy, or surgical resection). The cases were followed from date of diagnosis until either date of death or the last contact date for still alive patients.

Table 1. Distribution of clinical characteristics of treatment and survival in 176 glioblastoma patients from Sweden and Denmark

	Sweden (*n* = 108)		Denmark (*n* = 68)		Total (*n* = 176)	
	Total (*n* [%])	Missing (*n* [%])	Total (*n* [%])	Missing (*n* [%])	Total (*n* [%])	Missing (*n* [%])
Survival (mos)						
Mean	16.1		15.1		15.7	
Median (range)	12.4 (2.1–71.8)		14.2 (1.0–51.9)		12.8 (1.0–71.8)	
Gross total resection		6 (5.6)		1 (1.5)	169 (96.0)	7 (4.0)
Yes	26 (24.1)		24 (35.3)		50 (29.6)	
No	76 (70.4)		43 (63.2)		119 (70.4)	
Chemotherapy		13 (12.0)		10 (14.7)	153 (86.9)	23 (13.1)
Yes	69 (63.9)		14 (20.6)		83 (54.2)	
No	26 (24.1)		44 (64.7)		70 (45.8)	
Radiotherapy		7 (6.5)		2 (2.9)	167 (94.9)	9 (5.1)
Yes	90 (83.3)		57 (83.8)		147 (88.0)	
No	11 (10.2)		9 (13.2)		20 (12.0)	
Number of deaths	92 (85.2)	10 (9.3)	66 (97.1)		158 (89.8)	10 (5.7)
Sex					176 (100)	
Male	66 (61.1)		43 (63.2)		109 (61.9)	
Female	42 (38.9)		25 (36.8)		67 (38.1)	
Age at diagnosis median (yrs)	56 (22–69)		56 (39–69)		56 (22–69)	

SNP Selection

We accessed the SNPs selected for genotyping from the public dbSNP (http://www.ncbi.nih.gov), HapMap (http://www.hapmap.org/), and SNPper (http://snpper.chip.org/). Specific SNPs were chosen for genotyping in order to tag the linkage disequilibrium (LD) and to acquire genotype information for a larger number of SNPs within and surrounding the analyzed genes. A complete tagging of *EGF* and *EGFR* was based on the Haploview software (http://www.broad.mit.edu/mpg/haploview) with a minimum r^2 of 0.9. To ensure an LD to infer information on all SNPs captured by the tag set and to obtain a data set with sufficient power, we chose a minimum minor allele frequency of 5% in the HapMap CEPH (CEU) Utah residents with ancestry from Northern and Western Europe. DNA was extracted from samples using conventional methodologies and quantified using PicoGreen (Invitrogen Corp.).

Genotyping

Genotyping validation was first made on 14 family trios, in total 42 individuals, from whom genotype data are available through the HapMap consortium. Concordance analysis, using the HapMap data, and analysis of the parent–offspring compatibility with the produced genotypes were performed.

Genotyping was performed twice on 90 samples for all the SNPs in the study, as an internal concordance analysis. Three different DNA samples were used in quadruplicate on each 364-well plate that was analyzed. The success rate was calculated for each SNP genotyping assay as the number of genotypes retrieved over the possible number of genotypes. The concordance between the HapMap data and the data produced on the HapMap trios was 100% for all the SNPs. No parent–offspring incompatibility was found for any of the SNP data.

The concordance for the 90 repeated samples was 100%. The three repeated samples on all different analysis units showed concordant genotypes for all assays, at all instances when they were genotyped. The success rate for the different SNP assays spanned from 96.5% to 98%.

In all, 30 SNPs in *EGF* and 89 SNPs in *EGFR* were included in the genotyping analyzes, all passing the concordance and validation steps, and with a call rate at 80% or higher.

All SNPs were genotyped using matrix-assisted laser desperation/ionization time-of-flight mass spectrometry (Sequenom Inc.). Assays for all SNPs were designed using SpectroDESIGNER software (Sequenom Inc.). All SNPs were genotyped using the iPLEX assays. The amplification was performed in a total volume of 5 μL containing 10 ng of genomic DNA, 100 nM of each amplification primer, 500 mM of dNTP mix, 1.625 mM MgCl$_2$, and 5.5 units of HotStarTaq DNA Polymerase (Qiagen Inc.). The reaction was subjected to the following PCR conditions: a single cycle of denaturation at 95°C for 15 minutes, followed by 45 cycles at 94°C for 20 seconds, 56°C for 30 seconds, 72°C for 60 seconds, and a final extension at 72°C for 3 minutes. The allele-specific extension was performed in at a total volume of 9 μL using 5 pmol of extension primer and the Mass EXTEND Reagent Kit and cleaned using SpectroCleaner (Sequenom Inc.). Products from primer-extension reactions were loaded on a 384-element chip nanoliter pipetting system (Sequenom Inc.) and analyzed on a MassARRAY mass spectrometer (Bruker Daltonik GmbH). Data were analyzed independently by two persons using the SpectroTyper software (Sequenom Inc.).

The MDACC cases were genotyped in the Illumina 610k array and 4 of the 7 EGF polymorphisms were available on the 610k array for confirmation.

Statistical Methods

Statistical analyzes were undertaken by using SPSS software, and survival time was estimated as hazard ratios (HRs) for carrying different genetic variants in the *EGF* or *EGFR* gene using the Cox-regression model and having the major allele as the categorical variable. All HRs were adjusted for other factors that could affect glioblastoma outcome such as country, sex, age, surgery (gross-total resection or not), radiotherapy, and chemotherapy.

Results

We evaluated 30 tagging SNPs in *EGF* and 89 tagging SNPs in *EGFR* for association with survival in glioblastoma cases.

In the *EGF* gene, we found 7 SNPs associated with survival, 2 of the SNPs were located in the promoter region and the remaining 5 were located in introns 5, 7, 10, 11, and 13 (Table 2). In all these SNPs, there was an association with shorter survival except for the promoter SNP, rs6533477, where there were a heterozygote association with shorter survival and no homozygote variant for the minor allele. All 7 polymorphisms associated with poor prognosis are located on haplotype block 4 and are in LD with the previously identified functional polymorphism 61 A/G, *EGF* SNP rs4444903. The *EGF* SNP, rs3796944, had an HR of 2.93 (95% CI: 1.60–5.37) with a median survival time of 8.6 months for homozygote cases for the minor allele compared with 14.7 months for the major allele A.

Similar results with the minor allele associated with shorter survival could be seen in all SNPs when analyzing the Swedish and Danish data sets separately, see example in Table 3.

Four of the SNPs that were significant in EGF (rs17238095, rs3796944, rs9992755, and rs11568994) were available for confirmation in the MDACC patient cohort; however, none reached statistical significance (Table 4). In the whole data set, a nonsignificant trend of decreased survival was seen for rs3796944, the variant allele HR = 1.19 95% CI (0.94–1.51), with a 24.5 month median survival for the common allele and 18.5 months for the rare variant (Table 5). When comparing the characteristics of the 2 study groups, we saw a minor discrepancy in age at diagnosis (median

Table 2. SNPs in the EGF gene associated with glioblastoma prognosis in cases from the Swedish and Danish data set

rs number	Region	Major allele	Genotype	No. (%)	HR (95% CI)	P value	Median survival time
rs17238095	Promoter region	C	CC	77 (51.7)	1.0		14.4
			CT	67 (45)	1.37 (0.93–2.03)	.116	12.8
			TT	5 (3.4)	9.29 (2.35–36.78)	.001*	8.6
rs6533477	Promoter region	A	AA	133 (89.3)	1.0		13.2
			AG	16 (10.7)	2.09 (1.16–3.76)	.014*	11.7
rs3796944	Intron 5	A	AA	61 (40)	1.0		14.7
			AG	70 (45.8)	1.32 (0.88–1.96)	.177	12.8
			GG	22 (15.2)	2.93 (1.60–5.37)	.0005*	8.6
rs9992755	Intron 7	A	AA	72 (46.8)	1.0		14.7
			AG	70 (45.5)	1.45 (0.99–2.12)	.059	12.4
			GG	12 (7.8)	3.13 (1.47–6.65)	.003*	8.6
rs1860129	Intron 10	G	GG	60 (39)	1.0		14.7
			CG	72 (46.8)	1.30 (0.87–1.93)	.2	13.0
			CC	22 (14.3)	2.87 (1.57–5.24)	.001*	8.6
rs2298989	Intron 11	T	TT	56 (36.4)	1.0		15.1
			CT	73 (47.4)	1.47 (0.98–2.19)	.06	13.0
			CC	25 (16.2)	2.19 (1.24–3.88)	.007*	8.6
rs11568994	Intron 13	G	GG	73 (47.7)	1.0		14.7
			AG	67 (43.8)	1.38 (0.94–2.04)	.1	12.6
			AA	13 (8.5)	2.65 (1.28–5.51)	.009*	8.9

Patients diagnosed between 2000 and 2004, in Sweden and Denmark, as part of the Interphone study. All hazard ratios (HR) are adjusted for country, sex, age, surgery, radiotherapy, and chemotherapy. Number (No.) of cases analyzed varies due to missing treatment data or genotyping failure.
*Significant finding $P < .05$.

Table 3. Comparison between the Swedish and Danish results for the EGF SNP rs3796944 located in intron 5, major allele A

Country	Genotype	No. (%)	HR (95% CI)	P value
Sweden and Denmark	AA	61 (40)	1.0	
	AG	70 (45.8)	1.32 (0.88–1.96)	.177
	GG	22 (15.2)	2.39 (1.60–5.37)	.0005*
Sweden	AA	39 (44.8)	1.0	
	AG	35 (40.2)	0.92 (0.53–1.58)	.759
	GG	13 (14.9)	2.10 (1.02–4.32)	.044*
Denmark	AA	22 (33.3)	1.0	
	AG	35 (53.0)	1.62 (0.85–3.06)	.140
	GG	9 (13.6)	5.95 (1.89–18.77)	.002*

Hazard ratios (HR) are adjusted for country, sex, age, surgery, radiotherapy, and chemotherapy. Number (No.) of cases analyzed varies due to missing treatment data or genotyping failure.
*Significant finding $P < .05$.

Table 4. Validation on 4 of the significant *EGF* SNPs in a data set of 638 glioblastoma cases recruited at MDACC, from 18 years of age, recruited from 1990 to 2008, with follow-up until April 2009

	Region	Major allele	Genotype	No. (%)	HR (95% CI)	P value	Median survival time
rs17238095	Promoter region	C	CC	319 (54.1)	1.0		24.1
			CT	230 (39.0)	1.09 (0.93–1.31)	.632	23.4
			TT	41 (6.9)	1.28 (0.93–1.77)	.163	17.2
rs3796944	Intron 5	A	AA	200 (33.9)	1.0		24.5
			AG	284 (48.1)	1.09 (0.91–1.32)	.446	23.8
			GG	106 (18.0)	1.19 (0.94–1.51)	.231	18.6
rs9992755	Intron 7	A	AA	252 (42.7)	1.0		24.1
			AG	272 (46.1)	1.09 (0.90–1.28)	.561	23.1
			GG	66 (11.2)	1.06 (0.81–1.38)	.881	19.6
rs11568994	Intron 13	G	GG	251 (42.5)	1.0		24.2
			AG	274 (46.4)	1.16 (0.88–1.53)	.342	23.5
			AA	65 (11.0)	1.05 (0.87–1.25)	.876	18.1

All hazard ratios (HRs) are adjusted for sex and age.

Table 5. Validation analyses on 4 of the significant EGF SNPs in a data set of 374 glioblastoma cases recruited at MDACC, only including patients older then 50, recruited from 1990 to 2008, with follow-up until April 2009

rs number	Region	Major allele	Genotype	No. (%)	HR (95% CI)	P value	Median survival time
rs17238095	Promoter region	C	CC	192 (51.3)	1.0		18.9
			CT	156 (41.7)	1.19 (0.97–1.48)	.096	17.4
			TT	26 (7.0)	1.41 (0.95–2.11)	.088	15.0
rs3796944	Intron 5	A	AA	122 (32.6)	1.0		19.6
			AG	179 (47.9)	1.21 (0.96–1.52)	.096	17.8
			GG	73 (19.5)	1.32 (0.99–1.78)	.065	15.1
rs9992755	Intron 7	A	AA	150 (40.1)	1.0		18.1
			AG	183 (48.9)	1.10 (0.89–1.34)	.561	18.1
			GG	41 (11.0)	1.09 (0.79–1.55)	.741	17.4
rs11568994	Intron 13	G	GG	150 (40.1)	1.0		18.4
			AG	182 (48.7)	1.11 (0.90–1.37)	.448	18.1
			AA	42 (11.2)	1.24 (0.89–1.75)	.282	16.1

All hazard ratios (HRs) are adjusted for sex and age.

Table 6. SNPs in the EGFR gene associated with glioblastoma prognosis in the Swedish and Danish data set

rs number	Region	Major allele	Genotype	No. (%)	HR (95% CI)	P value	Median survival time
rs980653	Intron 1	C	CC	100 (66.2)	1.0		11.7
			CT	47 (31.1)	1.07 (0.99–1.14)	.079	14.2
			TT	4 (2.6)	0.60 (0.40–0.89)	.01*	9.2
rs730437	Intron 4	A	AA	45 (29.6)	1.0		10.8
			AC	67 (44.1)	0.53 (0.34–0.84)	.007*	13.2
			CC	40 (26.3)	0.50 (0.29–0.87)	.013*	15.1
rs4947986	Intron 7	G	GG	72 (47.7)	1.0		14.1
			AG	63 (41.7)	1.00 (0.67–1.48)	.98	13.2
			AA	16 (10.6)	2.21 (1.17–4.18)	.014*	9.9
rs9642393	Intron 19	T	TT	97 (64.2)	1.0		12.8
			CT	47 (31.1)	1.28 (0.86–1.90)	.226	13.2
			CC	7 (4.6)	3.44 (1.25–9.51)	.017*	9.6

Hazard ratios (HRs) are adjusted for country, sex, age, surgery, radiotherapy, and chemotherapy. Number (No.) of cases analyzed varies due to missing treatment data or genotyping failure.
*Significant finding $P < .05$.

56 years in our data set vs 54 in the confirmation set) and median survival time (12.8 months in our dataset vs 15 in the confirmation set) between the two groups with a younger age of diagnosis and longer survival time in the confirmation set. Since the survival was significantly longer in the MDACC data set, we assumed that one explanation for this could be a large amount of secondary glioblastomas among the younger population as the mean age differed between the populations. We therefore analyzed the SNPs using only the cases over 50 years of age in the confirmation dataset, leaving 374 cases to analyze, with 363 deceased in the group. Median survival time in the new group was 14 months and median age at diagnosis was 58 years. When restricting the analyses to cases 50 years or older to enrich for primary glioblastoma, the association of rs3796944 was marginally significant, HR = 1.32, 95% CI (0.99–1.79). The confirmation data set was adjusted for sex and age but not for treatment and surgery (Table 5).

In the *EGFR* gene we found 4 SNPs associated with survival, all located in different introns and haplotype blocks throughout the gene (Table 6). As 89 polymorphisms were tested in the gene, this is not more significant associations than would be expected by chance.

Discussion

This study is, to our knowledge, the first to perform a comprehensive tagging of the *EGF* and *EGFR* genes and estimates their prognostic impact, finding an association with EGF polymorphisms in haplotype block 4 and glioblastoma prognosis. Seven SNPs within the same LD block in *EGF* were associated with prognosis in glioblastoma cases with a consistent association in all the *EGF* genetic variants between the minor allele and poor prognosis. When analyzing 4 of the significant *EGF* SNPs in a larger confirmation set of 638 glioblastoma, we saw the same trend with minor allele association to shorter survival, but the results were only marginally significant when analyzing patients older than 50, which was performed to adjust for the discrepancies in age of the first and second data sets. The differences in the data sets could also, of course, depend on the variability of the data sets.

There have been only a few studies evaluating prognostic factors for glioblastoma. Previous studies have shown an association between young age at diagnosis, high Karnofsky performance score (KPS), and longer survival.[14] More frequent hypermethylation of the

MGMT gene has been found in glioblastoma patients with long-term survival, especially when treatment is given with temozolamide in combination with radiotherapy.[15] Although signaling pathways in glioblastoma are complicated networks, there are key factors such as dysregulation of growth factor signaling via amplification and mutational activation of receptor tyrosine kinase genes (eg, *EGFR* and *ERBB2*), activation of the phosphatidylinositol-3-OH kinase (*PI3K/AKT*) pathway and inactivation of the *p53* and retinoblastoma tumor suppressor pathways.[16]

A limited number of studies have investigated the role of *EGF* and *EGFR* polymorphisms and prognosis. Previous studies have been small and have not analyzed all of the tagging SNPs in *EGF* but have focused on one functional polymorphism, the 61 A/G. This polymorphism is situated in the 5′ untranslated region of the gene, where the uncommon 61G allele has a higher function for expression of EGF when compared with the 61A allele.[2] Higher levels of EGF have been associated with tumor progression and angiogenesis.[17] A small study of 42 glioblastoma patients showed that patients with AG and GG genotypes had higher levels of EGF in the tumor and were more likely to recur and progress.[1] In this study, they collected data on gender, age at diagnosis, surgery, and postoperative treatment (radiation- and chemotherapy) and found no difference in these variables when they compared cases by the different genotypes.[1] In a larger study of 197 glioma patients, with a mean age at diagnosis of 48.5 years, the *EGF* 61 A/G was associated with glioma risk, but not overall survival of glioblastoma patients; however, here the data are only adjusted for gender and age, without consideration of the extent of surgery or postoperative treatment.[7] One reason for the difference in results compared with our study could be the younger age in their study population. In a more recent study of 209 glioblastoma patients, the *EGF* 61 A/G polymorphism was found to be neither a clear prognostic factor for survival nor a risk factor for glioblastoma but with some impact on progression-free survival with a mean age of the patients similar to ours, 56.2 years.[2] In that study, adjustment for surgery and postoperative treatment was performed but not for radiotherapy and chemotherapy separately. The details of the types of chemotherapy were not given in the report. Thus, the results so far are unclear and inconclusive possibly because of lack of power and imprecise treatment information. The sample sizes are small, resulting in a lack of statistical power. In our study we observed a consistent association over the complete haplotype block harboring the *EGF* 61 A/G polymorphism. This is the most likely functional candidate genetic variant explaining the association with poor prognosis; however, other functional variants within the same LD block cannot be ruled out. Nevertheless, these results are in line with the proven functionality of increased EGF expression in blood and tumor observed in previous studies.[1,2] The impact of the polymorphism could be codependent on the existence of the *EGFR*vIII mutation, as the *EGF* signaling has less

impact on tumors with autophosphorylated mutations with autocrine loops for cell signaling.[18] The prevalence of *EGFR* mutations is not known in our data set or in the French study.[2] MDACC was unable to adjust for treatment, as we did with the initial 155 cases, but is still marginally significant in the patients older than 50 years. As the time period for recruitment is later in the MDACC, they might have a higher rate of temozolamide-treated patients. Further confirmation is necessary in separate data sets with similar treatment information to establish EGF as a prognostic factor.

Amplification and/or overexpression of *EGFR* have been found in about 50% of malignant glioma tumors; however, the prognostic value is unclear. One study has shown an association with better prognosis with *EGFR* expression when examining 403 cases with glioblastoma and adjusting for gender, age, and treatment. However, in the same study, they showed a significantly worse prognosis for astrocytoma with *EGFR* tumor overexpression. For both glioblastoma and astrocytoma, cases with EGFR overexpression were more strongly associated with survival than EGFR amplification.[10] In a small study of 41 glioblastoma patients over the age of 50 with available treatment data, no association between *EGFR* amplification and prognosis was found;[19] the same results were seen in a larger study of 715 glioblastoma cases.[20] In a group of 55 glioblastoma patients with long-term survival of more than 36 months, with treatment data available, a trend toward less *EGFR* amplification was seen; however, this was not statistically significant.[15] In a previous study from our group, we showed in small series of cases that low-grade gliomas with high EGFR protein expression had significantly shorter survival than those with no or low protein expression.[9] Therefore, tumor studies on EGFR have been inconsistent in prognostic relevance, and no studies, to our knowledge, have been published on the complete genetic variance of EGFR and glioblastoma prognosis.

Among our studied polymorphisms, the functionally most interesting and significant SNP in *EGFR* is rs4947986, located in the exon7/intron border. In 20–30% of glioblastomas, the extracellular part of the *EGFR* is mutated (*EGFRvIII*) with a deletion of exons 2–7, and the associated *EGFR* SNP rs4947986 is located at the break point of this common deletion, which could imply a functional role for prognosis in patients with glioblastoma. Some studies have identified Y845 as a phosphorylation site specifically activated by ionizing radiation.[21] Therefore, different genetic variants in *EGFR* could be hypothesized to have different functional properties in response to radiotherapy, which could give a functional explanation to differences in prognosis seen in our study. However, the associated SNPs in EGFR were similar to the estimated number of false-positive, leaving chance alone as the most likely explanation to the findings in EGFR.

This study provides some evidence for EGF polymorphism having a prognostic association for glioblastoma. Additional large studies with robust tagging of the complete gene variation of *EGF* and *EGFR* are

important to confirm previous observations with diverging results. As with all association studies, the consistency with functionality, results of previous studies, the power to detect association, and the possibility of false-positive findings must be taken into consideration. In this study, we have found an association of *EGF* and glioblastoma survival, with a clear functional genetic variation in the haplotype block and consistent with some,[1,7] but not all previous studies.[2] We observed a nonsignificant association in the same direction in the confirmation set; however, no treatment data were available to adjust for.

It is of importance to find robust genetic markers that could identify the right patients suitable for a specific treatment. A full comparison of the functional polymorphism in prospective studies with similar treatment given will elucidate the role for *EGF* polymorphisms as an independent prognostic factor.

Conflict of interest statement. None declared.

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