ORIGINAL PAPERS / ARTICLES ORIGINAUX

GLIOMA GENETIC SUSCEPTIBILITY AND SURVIVAL ANALYSIS IN THE EAST ALGERIAN POPULATION

SUSCEPTIBILITE GENETIQUE AU GLIOME ET ANALYSE DE SURVIE SUR UNE POPULATION DE L'EST ALGERIEN

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Mots-clés: *Algérie, Gliome, Polymorphisme, Susceptibilité*.
Key Words: *Algeria, Glioma, polymorphism, susceptibility.*

ABSTRACT

Background:

Genome-wide association studies (GWASs) have provided evidence for a polygenic basis of susceptibility to gliomas. The knowledge about these tumors remain very limited in Algeria. We aimed to investigate whether risk alleles identified by the GWASs were correlated with the glioma risk and the survival of patients in an Algerian cohort.

Methods:

We performed a case-control study on a cohort from east Algeria. We genotyped 17 polymorphisms identified by GWAS on the Sequenom MassARRAY platform. Fisher's Exact test was used to analyze the association between risk variants and glioma risk and Kaplan-Meier method for survival analysis.

Results:

We have found a negative association between the analyzed SNPs and glioma susceptibility. Even if not confirmed, our results revealed four interesting SNPs in our population: One variant (rs2736100, 5p15.33, TERT) has shown a protective effect (OR = 0.21; 95%CI [0.06-0.64], p = 0.006) and three were identified as prognosis factor, i.e. rs12076373, rs3751667 and rs10852606.

Conclusion:

The genetic composition of the Algerian population may therefore harbor specificities relative to the susceptibility to glioma and survival of patients not yet discovered.

RESUME

Description:

Les études d'associations à l'échelle du génome (GWAS) ont fourni des preuves d'une base polygénique de sensibilité aux gliomes. Les connaissances sur ces tumeurs restent très limitées en Algérie.

Objectif:

Nous avons cherché à déterminer si les allèles à risque identifiés par les GWAS étaient corrélés avec le risque de gliome et la survie des patients dans une cohorte Algérienne. Méthode : Nous avons réalisé une étude cas-témoin sur une cohorte de l'est Algérien. Dix-sept polymorphismes identifiés par les GWAS ont été génotypés sur la plateforme Sequenom MassARRAY. Le test exact de Fisher a été utilisé pour analyser l'association entre les variants de susceptibilité et le risque de gliome et la méthode Kaplan-Meier pour l'analyse de survie.

Résultats:

Nous avons trouvé une association négative entre les SNPs analysée et la susceptibilité au gliome. Même s'ils ne sont pas confirmés, nos résultats ont révélé quatre SNPs intéressants dans notre population : un variant (rs2736100, 5p15.33, TERT) a montré un effet protecteur (OR = 0,21; IC à 95% [0.06-0.64], p = 0.006) et trois ont été identifiés comme facteurs pronostiques, à savoir rs12076373, rs3751667 and rs10852606.

Conclusion:

La composition génétique de la population algérienne peut donc receler des spécificités non encore découvertes relatives à la susceptibilité au gliome et à la survie des patients.

INTRODUCTION

Gliomas are the largest group of Central Nervous System (CNS) tumors. Compared to the other cancers, they are relatively rare with a poor prognosis. They represent 1.6% of new cancers and 2.5% of cancer deaths worldwide (8). Genetic factors have been validated as important contributors for these neoplasms and recent genome-wide association studies (GWAS) have demonstrated that the inherited risk is due to the coinheritance of multiple low-risk genetic variants. So far, 25 risk loci have been identified as influencing risk (17,20,25). The GWA studies were carried on populations of developed countries (European and American). The Chinese population has revealed that not all the reported glioma risk–associated variants identified through GWAS are associated with glioma susceptibility (9,10). Data on developing countries remain very limited. That is the case of Algeria, where the genetic structure is highly heterogeneous (5) and the knowledge about gliomas remain scarce. Very few studies have been done and the limited budgets devoted to research in this developing country is one of the most limiting factors. Moreover, the lack of biological sample collection structure has driven researchers to focus much more on the most prevalent diseases.

The aim of this study was to investigate the glioma risk and the survival of patients in the east Algerian population based on the polymorphisms identified by GWASs.

SUBJECTS AND METHODS

2.1 Studied population

This case-control study involved 76 diffuse glioma patients and 82 apparently healthy individuals. Cases were recruited at the University Hospital Benbadis Constantine, where the majority of cancer patients from Eastern Algeria are received. Between March 2014 and October 2016, the researcher asked the patients about their willingness to participate in the study. Cases and controls were frequency matched on age and sex and they were from the same geographic region. The demographic and personal data were collected via a structured questionnaire. The clinical characteristics of the cases were obtained from medical records. Histopathological classification was based on the World Health Organization 2007 criteria (18). All the participants had no previous history of cancer and CNS-related diseases. Written informed consent was

obtained from each study subject prior to their participation in the study. The use of human blood sample and the protocol in this study strictly conformed to the principles expressed in the Declaration of Helsinki.

2.2 DNA preparation and SNP genotyping

Genomic DNA was extracted from peripheral blood samples using a standard salting out method (22). DNA concentration and purity were evaluated using a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA) and the PicoGreen technique specific to double strand DNA.

Seventeen of the Single Nucleotide Polymorphisms (SNPs) identified by GWASs with a Minor Allele Frequency (MAF) of at least 0.2 were selected for genotyping. Table 1 shows our selection of SNPs and their basic characteristics. Genotyping was performed on the Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA). Primers for polymerase chain reaction (PCR) amplification and single-base extension assays were designed using the Assay Design Suite V2.0 (Agena Bioscience™). The reactions were performed using the iPLEX™ Gold reagent kit. Data were collected with the Mass ARRAY System, a MALDI-TOF (Matrix-Assisted Laser Desorption Ionization – Time of Flight) mass spectrometer, and genotypes were analyzed using Sequenom Typer 4.0 Software. For quality control, 5% of samples were randomly selected, and the results showed 100% concordance.

2.3 Statistical analysis

Continuous variables were analyzed by Student *t* test and categorical variables by χ2-test. The Hardy-Weinberg equilibrium (HWE) was assessed for each SNP in controls. The correlation between polymorphisms and glioma development was estimated by Fisher's Exact test and the results were expressed by Odds ratio (OR) and 95% confidence interval (95% CI). Under an additive model, the Wilcoxon rank sum test was used to analyze the association between the total number of risk alleles and glioma susceptibility.

For survival analysis, the Kaplan-Meier method was used. The overall survival (OS) was defined as the time lapse from the day after surgery to the date of death or last contact. Univariate and multivariate analysis using the Log-rank test and the Cox proportional hazard model were performed to identify prognosis factors. $P < 0.05$ was considered as statistically significant. All analyses were conducted using R software (R-3.4.3).

RESULTS

Our analysis included 65 of our diffuse glioma patients and 72 of controls who were satisfactorily genotyped. The 17 SNPs tested were within HWE in controls.

3.1 Studied population

The demographic and clinical characteristics of patients and controls are reported in Table 2. Cases and controls were matched for age and sex, and there was no significant difference between them (p=0.64 and 0.78 respectively). The glioma group had a mean age of 50.3 years and was 66% male. Five of the patients had a low-grade glioma (grade II) and 60 had a high-grade tumor (grade III, n = 13; grade IV, n = 47). Glioblastoma was the most frequent histological subtype with 46 cases. Frequencies of the other types were as follows: astrocytoma (n = 5), oligodendroglioma (n = 6), oligoastrocytoma (n = 3), ependymoma (n = 3), gliosarcoma (n = 1) and glioma Not Otherwise Specified (n = 1). For statistical analysis, tumors were distributed as GBM or non-GBM. The gliosarcoma (grade IV) was integrated with the GBM group.

3.2 Case-control analysis

Table 3 shows the results of the correlation between our selection of polymorphisms and glioma risk in our cohort. In the analysis of each SNP, the rs2736100 at 5p15.33 in intron 2 of TERT gene was the unique polymorphism that has shown a significant p-value (p = 0.006), but it seems to have a protective effect (OR = 0.21; 95%CI [0.06-0.64]). The total number of risk alleles was not associated with the risk of glioma (p=0.38). After Bonferroni correction for multiple comparisons, the rs2736100 was no longer significant (p = 0.25).

3.3 Survival analyses

We found that patients aged ≥ 50 years had a 2.15-fold increased risk of death on overall survival (OS) (hazard ratio (HR) = 2.15, 95% CI: [1.19 – 3.88], p = 0.01) and that Non-GBM tumors were associated with 74% decrease in mortality hazard (HR = 0.26, 95% CI: [0.12-0.55], p = 0.0003) compared to GBM. The mean survival time of our cohort was 19.6 months, the median 12.2 months and 95% CI: [10.03-18.03].

In the univariate analysis, beside the age (p= 0.006), sex (p= 0.02), glioma subtype (p= 2e-04) and WHO grade (p= 0.001), three SNP were identified as prognosis factor, i.e. rs12076373 (p = 0.03), rs3751667 (p = 0.02) and rs10852606 (p = 0.03). However, in multivariate analysis none of them was confirmed. The total number of risk alleles was not associated with the survival of patients (p = 0.2) neither the rs2736100 (p = 0.8).

DISCUSSION

The genetic bases of GWAS, that have provided evidence for a polygenic susceptibility to glioma, are European and American (17,29,31,33,34,37,38). Studies on the Chinese population strongly suggested the important genetic heterogeneity in glioma risk (9,10). Within the North African context, the genetic composition of the Algerian population is an amalgam of different ancestral component coming from the middle East, Europe, sub-Saharan Africa and autochthonous to North Africa (Maghrebi) (3-5,13,15). With a such genetic wealth, data on glioma and rare diseases in Algeria and North Africa remain scarce because of the limited resources in biomedical research (16,32,36). To our knowledge, our study is one of the first investigations on glioma susceptibility in the region.

The risk of glial tumors in our population was associated with none of the 17 SNPs analyzed neither with the total number of risk alleles. Nonetheless, our results revealed an interesting polymorphism, the rs2736100 (5p15.33, TERT), which has shown a protective effect on glioma (OR = 0.21; 95% CI [0.06-0.64]) before the Bonferroni correction.

The risk locus 5p15.33 harboring the *TERT* gene, encoding the catalytic subunit of telomerase, has been implicated in several kinds of cancer (21,28) and the rs2736100, located in intron 2 of the *TERT*, has been linked to increased risk of glioma since the two first GWAS (33,38) and confirmed by several studies (40).

The absence of association between risk SNPs identified by GWASs and susceptibility to glioma as well as the protective effect of the rs2736100 in our cohort may be explained by ethnicity, since risk-allele frequencies correlate modestly between ancestry groups (23) and the identification of disease-associated SNPs by GWA studies tends to have low concordance when different populations are compared (39). Indeed, a same allele may be a risk factor in a population and a protective factor in another (12,35). Furthermore, many of the disease risk variants discovered by GWAS are shared across Eurasians, while the replication with individuals of African ancestry is much less common (19).

The survival analysis has shown that the overall survival in our cohort (mean 19.6 months and median 12.2 months) join other populations all over the world (1,2,7,24) as well as the known prognosis factors of glioma (i.e. age, sex, histological subtype and grade of malignancy) (6,14,24,27,30). Moreover, three SNPs (rs12076373, rs3751667 and rs10852606) may be associated with the survival of patients in our population, while a unique susceptibility variant (rs78378222) was associated with survival in GWAS (11). These associations, even if not confirmed, may indicate that variants identified by GWAS may be prognosis markers in our population. This is supported by the findings of Ostrom et al., who reported differences in incidence and survival of glioma by racial or ethnic groups (26).

Our results should be taken with caution since the modest sample size reduces considerably the power to detect all the susceptibility variants and prognosis factors in the Algerian population.

CONCLUSION:

In summary, our results indicate that germline risk variant of gliomas in our population may be different from those identified by GWASs and that some polymorphisms may be linked to the survival of patients. A larger sample is needed to identify the real glioma risk variants implicated in the Algerian population and sequencing the 5p15.33 region could reveal point mutations specific to this ethnic group.

Conflict of interest: The authors declare no conflict of interest.

Funding: This research was funded by the university of Constantine 1 Algeria.

I, the undersigned Sabrina TOUATI, first author, certify that all the persons cited have read and approved the mention of their name in the article

TABLES:

Table 1: Basic information on the 17 Single Nucleotide Polymorphisms (SNPs) analyzed

Locus	SNP	Gene	Base change	Glioma type
1q44	rs12076373	AKT3	G/C	non-GBM
2q33.3	rs7572263	near IDH1	A/G	non-GBM
3p14.1	rs11706832	LRIG1	A/C	non-GBM
3q26.2	rs1920116	TERC	G/A	High grade glioma
5p15.33	rs2736100	TERT	C/A	All
9p21.3	rs4977756	CDKN2B-AS1	G/A	All
10q24.33	rs11598018	OBFC1	C/A	non-GBM
10q25.2	rs11196067	VTI1A	A/T	non-GBM
11q21	rs7107785	MAML2	T/C	non-GBM
11q23.2	rs648044	ZBTB16	A/G	non-GBM
11q23.3	rs498872	PHLDB1	A/G	All
12q21.2	rs12230172	intergenic region	A/G	non-GBM
16p13.3	rs2562152	near MPG	A/T	GBM
	rs3751667	LMF1	C/T	non-GBM
16q12.1	rs10852606	HEATR3	T/C	GBM
20q13.33	rs6010620	RTEL1	A/G	All
22q13.1	rs2235573	SLC16A8	G/A	GBM

GBM: Glioblastoma

Table 2: Demographic and clinical characteristics of participants

Variable	Cases (n = 65)	Controls (n = 72)	P value
Age			
Mean ± SD	50.3 ± 12.9	49.2 ± 13.9	0.64
< 50	29 (44.6%)	34 (47.2%)	
≥ 50	36 (55.4%)	38 (52.8%)	
Sex			0.78
Male	43 (66.1%)	45 (62.5%)	
Female	22 (33.8%)	27 (37.5%)	
Histology			
GBM	47 (72.3%)		
Non-GBM	18 (27.7%)		
WHO Grade			
II	5 (7.7%)		
III	13 (20%)		
IV	47 (72.3%)		
SD: Standard Deviation			

Table 3: Odds ratios, 95% confidence interval and p-values of the 17 tested SNPs

SNP	OR1 (95%CI)	P1	OR2 (95%CI)	P2
RS12076373	1.43 (0.63-3.26)	0.39	0.58 (0.05-6.60)	0.66
RS7572263	1.29 (0.63-2.67)	0.48	2.10 (0.72-6.14)	0.17
RS11706832	0.68 (0.32-1.42)	0.31	0.88 (0.31-2.50)	0.81
RS1920116	1.90 (0.91-3.96)	*0.08*	1.32 (0.18-9.74)	0.78
RS2736100	0.82 (0.39-1.72)	0.59	**0.21 (0.06-0.64)**	**0.006***
RS4977756	1.91 (0.93-3.89)	0.07	0.90 (0.26-5.06)	0.87
RS11598018	1.58 (0.71-3.51)	0.25	0.97 (0.36-2.58)	0.95
RS11196067	1.19 (0.57-2.49)	0.63	0.64 (0.23-1.78)	0.39
RS7107785	0.66 (0.31-1.38)	0.27	0.65 (0.23-1.78)	0.40
RS648044	0.75 (0.35-1.59)	0.45	1.46 (0.43-4.99)	0.54
RS498872	0.99 (0.49-2.00)	0.97	0.66 (0.19-2.23)	0.51
RS12230172	1.20 (0.53-2.68)	0.66	1.10 (0.44-2.76)	0.83
RS2562152	1.17 (0.58-2.36)	0.65	4.55 (0.88-23.5)	0.07
RS3751667	0.88 (0.42-1.85)	0.73	2.24 (0.53-9.55)	0.27
RS10852606	0.56 (0.27-1.16)	0.12	0.35 (0.11-1.13)	0.08
RS6010620	0.54 (0.24-1.20)	0.13	0.46 (0.08-2.63)	0.38
RS2235573	1.17 (0.54-2.52)	0.69	0.65 (0.24-1.74)	0.39

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