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Interaction of allergy history and antibodies to specific Varicella zoster virus proteins on glioma risk

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Abstract

Glioma is the most common cancer of the central nervous system but with few confirmed risk factors. Glioma has been inversely associated with chicken pox, shingles, and seroreactivity to varicella virus (VZV), as well as to allergies and allergy-associated IgE. The role of antibody reactivity against individual VZV antigens has not been assessed. Ten VZV-related proteins, selected for high immunogenicity or known function, were synthesized and used as targets for antibody measurements in the sera of 143 glioma cases and 131 healthy controls selected from the San Francisco Bay Area Adult Glioma Study. Glioma cases exhibited significantly reduced seroreactivity compared to controls for six antigens, including proteins IE63 (OR = 0.26, 95%CI: 0.12-0.58, comparing lowest quartile to highest), and the VZV-unique protein ORF2p (OR = 0.44, 95%CI:0.21-0.96, lowest quartile to highest). When stratifying the study population into those with low and high self-reported allergy history, VZV protein seroreactivity was only associated inversely with glioma among individuals self-reporting more than two allergies. The data provide insight into both allergy and VZV effects on glioma: strong anti-VZV reactions in highly allergic individuals is associated with reduced occurrence of glioma. This result suggests a role for specificity in the anti-VZV immunity in brain tumor suppression for both individual VZV antigens and in the fine-tuning of the immune response by allergy. Anti-VZV reactions may also be a biomarker of effective CNS immunosurveillance due to the tropism of the virus.

Keywords

glioma; varicella; serology; case-control; allergy

Introduction

Glioma was diagnosed in over 237,000 people in the world in 2008.[1] Glioma has relatively few known risk factors, apart from ionizing radiation and a small number of confirmed inherited genetic variants.[2] Infections and immune factors are thought to impact

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gliomagenesis; the brain hosts certain types of immune reactions and microglia cells are the resident macrophages in the brain.[3]

A potential viral etiology for brain tumors has long been suggested, starting with the investigation of the Simian polyomavirus SV40 which was a contaminant of polio vaccinations.[4] While little evidence for polyomaviruses like SV40 has emerged, more recent studies imply a role for herpesviruses, primarily cytomegalovirus (CMV) and varicella virus (VZV). CMV is present as an active virus in most human gliomas and has some biological characteristics suggesting that it may contribute to the malignant phenotype.[5] CMV remains under investigation as a tumor-promoting virus; however, the serological response to CMV is not significantly different in glioma patients compared to healthy controls.[6] Anti-VZV IgG, on the other hand, was reported to have lower levels among glioma cases compared to controls, providing support that a serological response to VZV may be protective.[6-8] Although the nature of this protective effect is not yet known, a recent study showed that the VZV virus can infect glioma cells, and this infection enhances T-cell mediated cytotoxicity.[9] This suggests that VZV antigen(s) may produce protein epitopes which are cross-reactive with glioma antigen(s).[9] The presence of VZV viral particles within human gliomas has not been reported.

VZV is a DNA-based alphaherpesvirus containing 71 open reading frames, 68 of which encode proteins and thus may provide immune targets.[10] Like Herpes simplex 1 and 2, VZV infects mucoepithelial cells and following the clearing of disease (for VZV, chicken pox), VZV establishes latency in neurons. Individuals are typically exposed to VZV in early childhood, and the virus establishes long-term latency in dorsal root nerve ganglia. Reactivation of the virus often occurs in their fifth and sixth decades, leading to zoster (shingles).[11]

Along with viruses, increasing interest has focused on immune factors centered on allergies, which have been consistently reported to be less common in individuals who develop glioma compared to controls [reviewed in [12, 13]]. Further, allergy-associated IgE and other allergy-related biomarkers also are inversely associated with glioma, even when assessed years prior to diagnosis.[14-17] Our current study follows-up on our and others VZV and allergy-related IgE findings with a focus on two concepts: (i) Are their specific anti-VZV antibodies that are associated with a reduced risk to glioma? (ii) Is there a joint effect between prevalence of anti-VZV antibodies and history of allergy on glioma risk? To address these questions we used a panel of serological targets to VZV and the biological and data resources of the San Francisco Bay Area Adult Glioma Study (SFBAGS).

Materials and Methods

Study participants

Histologically confirmed glioma patients (International Classification of Diseases for Oncology, morphology codes 9380-9481) diagnosed from November 1991 to September 1999 (before the widespread introduction of temozolomide chemotherapy) were identified using the Northern California Rapid Case Ascertainment program. Eligible cases for the current analysis were aged 20 or older, white, no history of temozolomide exposure, and resided in the 6 county San Francisco Bay Area, (Alameda, Contra Costa, Marin, San Mateo, San Francisco, and Santa Clara), and had a blood specimen. All glioma diagnoses were reviewed by an academic neuropathologist as previously described.[18] Controls aged 20 years or older from the same residential area as cases were identified using random digit dialing and were frequency matched to cases by age (five year groupings), gender and ethnicity. In-person interviews were conducted with cases (or their proxies) and controls, and allergy history assessment questions were asked as detailed in our earlier manuscript on

the association between allergies and glioma risk in the same study population.[18] This allergy assessment was a detailed history collected in tabular form on 6 questionnaire pages. Prevalence of allergy (respiratory, dermal, and food) was 85% among controls as described previously [18]; true allergies are likely to be present in those who exhibit multiple allergies.

Blood and sera were collected either at the time of interview or at a later time; on average about 4 months post-surgery for cases. At the time of venipuncture, participants were asked about currently used medications and recent chemo- and radiation therapy. Three hundred and forty-seven cases and 347 frequency-matched controls met the criteria for this sub-study with 143 cases and 131 controls randomly selected for these analyses. Data available for these individuals included serum IgE level, total serum anti-VZV level [assessed previously by ELISA assay using total VZV antigen [6]], specific SNPs in chr9 that have been associated with allergies and glioma risk (rs1063192, rs1412829, rs2157719 and rs4977756), tumor *IDH1/2* mutation status and allergy history.

Antigen Selection and Protein Synthesis

We selected VZV antigens with reportedly high seroprevalence in general population [19] and/or those with well-established biological functions [20, 21] from among the 68 known VZV proteins. Target proteins include glycoproteins E, H, I and K (gE, gH, gI and gK, respectively) and orf2, orf9, orf12, orf20, orf24, and orf26 proteins (ORF2p, ORF9p, ORF12p, ORF20p, ORF24p, and ORF26p, respectively) and two immediate early proteins, IE62 and IE63. The names and roles of ten VZV antigens successfully analyzed are presented in Table 1. Three proteins, gE, ORF9p and ORF26p were purchased from Fitzgerald Industries, Inc. (Concord, MA). For the remaining 9 proteins, gene synthesis and protein expression were performed through the Assembly PCR and cell free *in vitro* Wheat Germ Protein Expression System from Abnova (Taipei City, Taiwan).[22]

Luminex VZV Antibody Assay

Serum samples collected from each participant and stored in −70°C were tested for their antibodies against the different VZV antigens using the Luminex assays, a sandwich capture method.[23] All experiments were done using the Bio-Plex™ Amine Coupling Kit and Bio-Plex Pro™ Magnetic COOH Beads (Bio-Rad Laboratories Inc., Hercules, CA). Briefly, synthesized VZV antigens were coupled to carboxylated microspheres (beads) by using a two-step carbodiimide reaction. For multiplexing, the microspheres were designed to have different internal fluorescent dyes matched to different VZV antigens. Test sera sets were used to determine the titer and optimal dilution of sera. Antibody titers ranged from 1:1000 to 1:10,000, and a 1:100 dilution sufficed for all antigens except for two. Bead sets were incubated in pairs, triplex, and with all bead sets together with test sera to determine whether any assay interacted or affected the result of other assays when used in combination. Interactions were not demonstrated, and all bead sets were used together.

Serum samples were diluted 1:100 in duplex in a diluent made of PBS, 10% fetal bovine serum, and 2.5% CBS-K (Millipore Corporation, Billerica, MA). The diluted sera and coupled microspheres were co-incubated for 2 hours at room temperature on a shaker. After washing, a secondary antibody (Goat anti human IgG (H+L)-biotin, Southern Biotech 2085-08) was added and incubated for 30 minutes. The solution was then treated with streptavidin-conjugated R-phycoerythrin. After 10-minute incubation, microspheres were resuspended in 105 μL of assay buffer. The amount of antibodies bound to the microspheres was determined by the median fluorescence intensity of the reporter molecule, phycoerythrin, using the Bio-plex 200 plate reader system (Bio-Rad Laboratories Inc., Hercules, CA). Cases and controls were randomized prior to analysis, and each plate was assayed with no more than 10% difference in numbers of cases and controls. All samples

were assayed in duplicate (technical duplicate) and when coefficient of variation was more than 20% the assay repeated. Duplicates were averaged for further analysis.

Statistical Analysis

All statistical analyses were conducted using SAS v9.3 (Research Triangle Park, NC). Continuous measures (log transformed anti-VZV analytes, VZV antibody and IgE levels) also were analyzed grouped in quantiles based on the distribution among controls. Preliminary analyses of the association among analytes, covariate factors and disease status were assessed using Wilcoxon rank sum for continuous variables, and Fisher's exact or Chi-square statistics for categorical data. Correlation between analytes and other covariates also were determined by disease status using Spearman's rank correlation. Analyte levels were evaluated in age and sex adjusted logistic regression models that used cluster methods to account for between plate differences. Odds ratios and 95% confidence intervals for VZV analytes were computed from these models as estimates of the relative risk. Effect modification was evaluated in analyses stratified by sex, allergy history, median number of allergies, median IgE level, and median VZV level with formal tests of statistical interaction based on the Wald chi-square statistic for the cross-product term of a specific analyte and covariate of interest. Potential confounders including education, smoking status, history of allergies, median number of allergies, previously measured total VZV antibody level, current dexamethasone use, and IgE level were included in the final parsimonious models if they altered the odds ratio estimate by >10%. Analyses also were conducted for a composite analyte score that was computed as the sum of the number of analytes that were greater than the median for each individual. Independence of analyte effects were assessed in an adjusted logistic regression model that conditioned on all analytes.

Results

Examination of the distribution of analyte values showed good performance with the exception of two analytes, ORF9p and IE62, where results suggested “over-saturation” with most values clustered at fluorescent values at the upper limits of photomultiplier detection. Therefore we excluded these two analytes from further analyses. The distribution of all other anti-VZV antibody measures were right tailed, and approximately normal when log-transformed.

As shown in Table 2, cases and controls were similar for age, years of education, percent men, and cigarette smoking status (Table 1). Total serum anti-VZV levels (to the whole virus) was previously assessed in these participants [6]. In this small participant sample, cases had lower median value than controls, but the difference was not significant (Table 2). Antibodies to Cytomegalovirus, Herpes Simplex I, and Epstein-Barr viruses were also assessed in these participants previously, and in the current sample (like the larger published dataset, [6]), these measurements did not differ between cases and controls (P = 0.8, 0.2, and 0.2 for the three viruses, respectively, when comparing positive to negative measurements by χ^2 test). A greater proportion of controls reported a history of allergies and controls were more likely to have higher IgE level than cases [as previously shown, [15]].

Interestingly total anti-VZV (assessed previously, [6]) was correlated with only two out of ten of the individual anti-VZV analytes, gI (rho=0.28, controls only) and ORF24p (cases, rho=0.20; controls, rho=0.18) (Supplemental Table 1). A number of analytes were correlated with any allergy history as well as with number of self-reported allergies. These correlations were generally inverse between cases and controls: negative among cases and positive in controls. Also, correlations were more likely to be statistically significant among controls than cases although statistically significant correlations between number of allergies and

IE63 were observed both for cases (rho=-0.18, p=0.046) and controls (rho=0.35, p<0.0001, Supplementary Table 1).

IDH1/2 mutation status was available for 116 of the 143 cases in our VZV analyte assessment. Only one analyte was associated with *IDH* status; a positive correlation was observed with ORF12p (p=0.007) (Supplement Table 1). For the four chr9 SNPs evaluated, no associations with anti-VZV analytes were observed among controls. Several borderline (p<0.10) positive correlations were observed in cases although only the positive correlation between rs1063192 and ORF26p was statistically significant (rho=0.17, p=0.04).

Analyte levels were grouped into quartiles based on the distribution in control participants. Regardless of level or of specific analyte, increasing levels were associated with decreased occurrence of glioma (Table 3) and the trends were statistically significant for 6 of the 10 analytes (gH, IE63, gE, ORF26p, ORF2p, ORF20p; all p for trend <=0.05). Monotone trends were exhibited for anti-ORF20p and IE63. There was a borderline decreased risk observed for a seventh (ORF12p, p for trend=0.07). Median number of reported allergies (≤2, >2) and smoking status attenuated the associations of glioma to specific analytes and therefore are included in the final parsimonious models as appropriate (noted in footnote in Table 3). Overall, those with analyte levels in the highest quartile were 45-84% (depending on the specific analyte) less likely to have glioma than were those with levels in the lowest quartile (Table 3).

In age and sex adjusted logistic regression analyses that included clustering by plate, mutual adjustment for analytes as log transformed continuous variables showed that ORF2p, IE63, gH and ORF12p were independently associated with glioma (all p <0.02, data not tabled). Consistent with main effect results, increased levels of ORF2p, IE63, and gH were associated with decreased glioma occurrence; however, the odds of glioma increased 27% for each unit increase of ORF12p. Analysis of glioma associations by sum of the number of analytes above the median yielded an OR = 0.91 (95% CI 0.86-0.95, P < 0.0001, data not tabled).

Results of analyses by sex (Table 4A) demonstrate significant interactions. Decreased occurrence of glioma with increased levels of analytes was observed for gI and gK among women and IE63 and ORF12p among men (p interaction: <0.0001, 0.04, 0.003, 0.01 respectively). This result was not driven by gender differences among controls, as there were no significant differences in anti-VZV antibody levels between genders in controls (Wilcoxon rank test, P values range from 0.11 to 0.99).

We chose a cutoff of “3 or more” allergies to describe an allergic person. This choice was motivated by our previous results which indicate that the strongest case-control odds ratios were achieved with more than 2 allergies.[18] Also, our allergy “prevalence” is about 85% among controls, which is much higher than the 30-40% commonly found in formal studies on allergy prevalence. This high prevalence is likely due to our 6-page extensive questionnaire on allergy symptoms, which are supplied by self-report rather than physician diagnosis. Results by median number of allergies (Table 4B) also demonstrate significant interactions. For gE, ORF2p, ORF12p, ORF20p, ORF24p and IE63, decreasing association with increased analyte levels were observed only among those with >2 allergies, all p interaction ≤0.02). For total serum anti-VZV IgG, a decreased trend was observed only among those with higher median total anti-VZV IgG and only for two specific analytes (gI, p interaction=0.03; gH, p interaction=0.02, data not shown). There was no evidence of an interaction between median IgE level and any analyte (data not shown).

Dexamethosone is commonly used in glioma treatment, and 43% of this case group (59 of 136 patients) was under current treatment with dexamethasone at the time of blood draw. Only one control was taking the drug. Dexamethosone was significantly inversely associated with several anti-VZV targets including ORF2p, ORF20p, ORF24p, and ORF26p (P = 0.003, 0.01, 0.01, and 0.01 respectively) when comparing numbers of case participants in quartile levels of anti-VZV antibodies among those who were taking, and not taking, dexamethosone (by χ^2 test). To account for this, we calculated case-control odds ratios stratifying the case group into those who did and did not take dexamethasone, and comparing both to the overall control group (Supplementary Table 2). Tertiles were used due to the smaller sample sizes in the two strata. Odds ratios were slightly attenuated by dexamethosone treatment, indicating either that the drug or the indication for the drug might have an effect on anti-VZV antibody levels; however, confidence intervals still excluded 1 for several anti-VZV antibodies in the non-dexamethosone group including those for gE, gH, and ORF12p (Supplementary Table 2). Odds ratios for most other analytes were in a similar direction in the dexamethasone strata compared to those not currently treated with dexamethasone, though not significant for the latter.

Discussion

In addition to the well-established inverse correlation between allergy or immunoglobulin E levels and glioma risk,[12-16, 24] anti-VZV immunity is also clearly associated in the similar inverse direction. In a comprehensive analysis of familial and personal medical histories in adults with glioma, we previously showed that history of chickenpox and/or shingles was inversely correlated with case status.[25] Subsequent serologic studies for four neurotropic herpesviruses, herpes simplex virus (HSV), cytomegalovirus (CMV), VZV and Epstein-Barr virus (EBV), showed that only VZV-specific immunoglobulin levels were significantly and inversely associated with glioma risk.[6-8] To obtain more detailed biological insights, the current study analyzed glioma patients' serological responses against 10 different VZV proteins. Accordingly, antibodies to viral proteins showed strong inverse yet individually variable correlations with glioma risk, suggesting a connection of anti-VZV immunity to cancer development. Associations to individual proteins did not specify a single immunodominant epitope when assessed over our study population; but rather, several particularly strong associations to key VZV proteins and strong interactions of anti-VZV responses with self-reported allergy but not IgE levels.

In our final model, antibodies to IE63 was the most significant marker associated with glioma status ($P<0.0001$). The biological function of this or any other individual VZV protein is not implied to be linked to their immunogenicity, but may help instruct mechanisms behind the different immune responses of glioma patients from controls. IE63, an immediate early protein related to transcriptional activation during viral entry and replication, is the only protein proven to be essential for the establishment of latency, among the many VZV proteins studied so far.[20, 26-29] It is required for viral immune evasion by suppression of interferon-α induced host antiviral response.[30] Interestingly, IE63 also suppresses apoptosis of neurons.[31] We also observed strong correlations of gE and gH with glioma, especially in men and in patients who reported more than 2 allergies. Of the seven membrane glycoproteins, gE is most abundantly expressed and thus highly immunogenic.[32] Expressed in infected host cell membranes, gE plays a key role in cell fusion and viral propagation [33, 34] and is indispensible for viral infectivity and growth.[35-37] VZV gH is also a major VZV fusogen required for endocytosis of viral particles.[38, 39] Furthermore, gH contains an immunodominant complement-independent neutralization epitope, where directed antibodies can effectively block viral entry and spread.[40-42]

We could not find any significant association of gI, except for a borderline association in women. VZV gI forms a heterodimer with gE, enhancing its activity,[43] but is found to be dispensable for viral growth or establishing latency.[35, 44] Another glycoprotein, gK was insignificant in the parsimonious model but was significantly associated in women. This protein is less well studied, but one investigation found that gK may be essential for viral syncytia formation and growth.[45] ORF2p and ORF20p were significant both in the parsimonious model and stratified analyses, while ORF12p was significant only in stratified analyses. While ORF2p and ORF12p are dispensable for *in vitro* growth;[46-48] more relevantly, the high seroprevalence of antibodies to these proteins [19] suggests a possible link to host immune response. For the other less-well characterized proteins, ORF24p and ORF26p, negative to borderline levels of significance were reached, suggesting possible additive or bystander roles in immune responses.

This study has several weaknesses as well as strengths. First, we have assayed glioma cases after diagnosis, and gliomas and treatments for gliomas are known to suppress the immune system. We do note however that antibody levels other herpesviruses (anti-CMV, HSV1, and EBV) were not reduced in cases compared to controls. Also, our previous studies on other antibody markers did not detect effects on antibody levels from chemotherapies, comorbidities, time of day, season, or time since surgery.[15] We did however detect effects of temozolomide treatment here,[24] and therefore we excluded cases treated as such for the current analysis. Since temozolomide was introduced as “standard of care” over 10 years ago, our samples tested were older samples (pre-2000), with possibly some degradation of antibodies. We were however unable to detect differences in antibody levels by age of sample (data not shown). Dexamethosone here was also shown to affect specific antibody levels, an effect which was not detected in prior studies on other antibodies.[6, 7] This affect attenuated some case-control differences, suggesting that prediagnostic or at least pre-treatment sera would be a better medium for assessing viral antibodies. Another potential weakness is related to sample sizes which are not large, leading to potentially spurious results particularly in subgroup analysis. For this reason, we cannot make any firm conclusions about gender effects despite many strong interactions. The consistency of the allergy/anti-VZV antibody interaction results, as well as clear variability among the various antigens, argues in favor of these results not being explained by glioma- or treatment-related effects or spurious variation. Another potential weakness is the apparent lack of interaction with IGE levels while self-reported allergy interaction is strong. While IGE levels are correlated with one type of allergy pathology, *i.e.*, atopy, IGE levels are not an accurate assessment of allergy or a more definitive marker of the allergic pathology that affects glioma risk when compared to self-reported allergy.[15, 24] Self-reported allergy may better capture a lifetime exposure to allergic pathologies that are relevant to glioma risk. Finally, our study was only able to capture a single blood sample from cases and controls, and did not establish the longitudinal variability of antibody measurements. While some variability is certain to be the case, varicella antigens are internally sourced and likely somewhat constant immune stimulants. Also, the relative differences between groups of people should be valid even if the absolute titers in individuals will vary over time.

Several hypotheses and issues could be raised for the underlying mechanisms of the association between anti-VZV immunity and glioma development. As a latent virus, VZV has an intrinsic potential to modulate tumor development through chronic infection and inflammation.[11, 22] Cross-reactiveness between VZV and glioma cells was also suggested because VZV-stimulated cytotoxic T lymphocytes (CTLs) selectively kill glioma cells.[9] Our results may favor the former hypothesis, i.e. tumor predisposition from chronic infection and inflammation, because the most significantly associated antigens are those critical for virulence. The varied response to VZV antigens is also likely to be related to individual variability in epitope presentation based on HLA variability. Future studies should consider

anti-VZV antibody and T-cell responses in concert with the presentation of specific epitopes by HLA status.

VZV shares many homologous proteins with other herpes viruses, especially HSV. One protein, ORF2p, is unique to VZV with no homology to other HSV proteins (Table 2).[21] This protein is strongly associated with case status, confirming a true association of VZV rather than other cross-reacting viruses. While we cannot rule out specific proteins as glioma epitopes, anti-VZV antibodies measured here could serve as a biomarker of the presence of strong CNS immunosurveillance mechanisms, a kind of immunologic beacon based on VZV's tropism to the CNS. The attraction of immune activity to the CNS by VZV may impart collateral protection from brain cancer, especially in persons with strong allergic phenotype-biased immune systems.

Cellular immunity by anti-VZV CTL (which kill infected cells) is known to play a principal role in the defense against viruses. Humoral immunity, which the current antibody study is directed, appears to play a minor role in controlling the virus.[11, 20] We found significant associations only in hyper-allergic individuals (defined as number of allergies >2) whose humoral immunity is much enhanced.[49] So it could be postulated that VZV antibodies are more effective and have a synergistic role in these “humorally-skewed,” individuals. We note that the brain is an immune-privileged site where immune mechanisms differ from peripheral tissues. The dominant mechanisms of viral clearance in brain appear not to be CTL-dependent cytolysis; but rather, mediated by complement-independent neutralizing antibodies or T cell cytokines such as interferon suppressing viral replication, to reduce inflammation and parenchymal damage.[50] The humoral dominance of immune reactions in the brain could favor anti-tumor immunity in individuals with a T-helper-2 type immune dominance, a hypothesis suggested by ours and other's observations that individuals with strong allergies have lower brain cancer risk.[12, 13] CTL-dependent cytolysis is still important as a first-line defense against VZV viral entry and propagation in skin mucosa and blood lymphocytes,[11, 20] but increased VZV antibodies and enhanced humoral immunity may be more beneficial for long-term anti-glioma effects.

We also observed different associations according to gender. Antibodies to IE63, gE, gH and ORF12p were very significant in men, whereas those to gK, ORF2p and ORF20p were so in women (Table 4). The reason is unclear but it might be from the inherent differences in immune systems between men and women; both cellular and antibody-mediated immune responses are typically higher in women than men.[51] Furthermore, women are more prone to T-helper-2 cytokine profiles related to humoral immunity.[52] Such different immune profiles might lead to the gender differences in antigen recognition and effectiveness.

In summary, we studied associations of antibody levels to specific VZV proteins with glioma. Antibodies to proteins important for VZV virulence including IE63, gE and gH were among the most significantly associated with glioma case status, as well as ORF2p and ORF20p, suggesting a pivotal role of humoral immunities to these proteins. Such associations were only prominent in hyper-allergic patients whose humoral immunity is higher than in less allergic people. We also found a gender difference in associated antigens suggesting that different immune mechanisms may underlie differences in glioma risk among men and women. Our results require confirmation in additional larger studies with more anti-VZV markers but provide intriguing and compelling evidence on the relationship between anti-VZV immunity and glioma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Ten open reading frame (ORF) antigens and related protein role; selected from among 71 open reading frames that encode 68 genes in the VZV genome for analysis in 143 glioma patients and 131 frequency-matched controls, San Francisco Bay Area, Adult Glioma Study. “Essential” means that this protein is required for that function, “Dispensable” means that it is not.

VZV antigen	Gene	HSV-1 homolog	Name/role	Viral replication	Establishment of latency	Reference
gK	orf5	UL53 (gK)	Glycoprotein K	Essential	-	[45]
gH	orf37	UL22 (gH)	Glycoprotein H	-	-	
gI	orf67	US7 (gI)	Glycoprotein I	Dispensable	Dispensable	[35, 44]
gE	orf68	US8 (gE)	Glycoprotein E	Essential	-	[35-37]
ORF2p	orf2	None	Membrane phosphoprotein	Dispensable	Dispensable	[46, 47]
ORF12p	orf12	UL46 (VP11/12)	Tegument protein/transactivator	Dispensable	-	[48]
ORF20p	orf20	UL38 (VP19C)	Capsid protein	-	-	
ORF24p	orf24	UL34	Membrane protein	-	-	
ORF26p	orf26	UL32	Virion/packaging protein	-	-	
IE63	orf63	US1 (ICP22)	Tegument protein/transactivator	Essential	Essential	[26-28]

Table 2

Demographic characteristics and descriptive factors of white glioma cases and controls, San Francisco Bay Area Adult Glioma Study (AGS)

Factor	controls N=131		cases N=143		p-value[a]
Age in yrs (mean, standard error)	53.2, 1.30		52.8, 1.27		
ln IgE (median, IQR[b])	3.52, 2.04		2.80, 2.12		0.001
ln VZV (median, IQR[b,c])	0.41, 1.28		0.31, 1.44		0.16
	n	**(%)**	**n**	**(%)**	
Sex					
Women	52	40	58	41	
Men	79	60	85	59	
Education Highest Degree					
HS	39	30	50	35	
Jr College	18	14	23	16	
College	48	37	42	29	
Graduate Degree	26	18	28	20	
					0.59
Cigarette smoking					
Never	54	42	59	41	
Past	52	40	58	41	
Current	24	18	25	18	
					0.98
Any allergy					
no	22	17	43	30	
yes	108	83	100	70	
					0.01
Median # of allergies					
≤2	66	51	89	69	
>2	64	49	40	31	
					0.003
Median VZV[c] (raw level)					
<1.5	65	50	80	56	
1.5+	66	50	63	34	
					0.29
Median IgE (raw level)					
<33.9	65	50	92	64	
33.9+	66	50	51	36	
					0.01
Histology					
Glioblastoma			89	62	

	n	(%)	n	(%)
Non-GBM (Grade II & III)			60	38
IDH1				
WT			82	70
Mutated			34	29
Inconclusive			1	1

Numbers may not sum to total due to missing values

[a] p-value from Wilcoxon test (IgE and VZV), from Fisher's exact (any allergy) and from Chi-square (education, smoking); not presented for age and sex as these were matching factors

[b] IQR - Interquartile range. Ln(IGE) and ln(VZV) levels were previously determined (reference #9 and 4 respectively).

[c] Total VZV measured by ELISA and was previously assayed and reported [6].

Table 3

Adjusted odds ratios (OR) and 95% confidence intervals (CI) for glioma associated with levels of specific VZV analytes grouped by quartiles, San Francisco Bay Area Adult Glioma Study

Quartile Groups	Cases (N=143)	Controls (N=131)	OR, 95%CI[a]
gK[c]			
<8.03	56 (39) [b]	33 (25)	ref
8.03-<8.67	23 (16)	33 (25)	0.35, 0.17-0.72
8.67-<9.66	25 (17)	31 (24)	0.46, 0.20-1.08
9.66+	39 (27)	34 (26)	0.66, 0.40-1.09
P for trend			*0.23*
gH[c]			
<7.88	58 (40)	33 (25)	ref
7.88-<8.12	27 (19)	33 (25)	0.39, 0.25-0.59
8.12-<8.89	46 (32)	32 (24)	0.67, 0.35-1.30
8.89+	12 (8)	33 (25)	0.16, 0.08-0.32
P for trend			*0.0003*
gI[d]			
<8.15	39 (27)	33 (25)	ref
8.15-,9.06	43 (30)	32 (24)	1.08, 0.74-1.58
9.06 - ,9.98	32 (22)	33 (25)	0.79, 0.48-1.32
9.98+	29 (20)	33 (25)	0.71, 0.31-1.64
P for trend			*0.36*
gE[c]			
<8.16	51 (36)	31 (24)	ref
8.16-<8.6	31 (22)	34 (26)	0.59, 0.34-1.05
8.6-<9.1	26 (18)	33 (25)	0.44, 0.26-0.72
9.1+	35 (24)	33 (25)	0.55, 0.33-0.90
P for trend			*0.0002*
ORF2p[c]			
<7.52	59 (41)	33 (25)	ref
7.52-,7.96	34 (24)	33 (25)	0.56, 0.32-0.98
7.96 -<8.41	19 (13)	32 (24)	0.30, 0.21-0.42
8.41+	31 (22)	33 (25)	0.44, 0.21-0.96
P for trend			*0.001*
ORF12p[e]			
<7.33	45 (31)	33 (25)	ref
7.33-<7.88	42 (29)	32 (24)	1.04, 0.40-2.69
7.88-<8.57	29 (20)	34 (26)	0.56, 0.31-1.02
8.57+	27 (19)	32 (24)	0.47, 0.15-1.46
P for trend			*0.07*

Quartile Groups	Cases (N=143)	Controls (N=131)	OR, 95%CI[a]
ORF20p[c]			
<7.29	55 (38)	33 (25)	ref
7.29-<7.61	32 (22)	32 (24)	0.56, 0.28-1.13
7.61 -<8.12	28 (20)	33 (25)	0.44, 0.23-0.85
8.12+	28 (20)	33 (25)	0.42, 0.23-0.79
P for trend			*0.0009*
ORF24p[a]			
<8.03	48 (34)	33 (25)	ref
8.03-<8.42	29 (20)	33 (25)	0.60, 0.38-0.95
8.42 -9.04	31 (22)	32 (24)	0.66, 0.36-1.21
9.04+	35 (24)	33 (25)	0.73, 0.35-1.51
P for trend			*0.46*
ORF26p[e]			
<8.40	53 (37)	33 (25)	ref
8.40-<8.68	30 (21)	32 (24)	0.50, 0.20-1.26
8.68-<8.94	30 (21)	33 (25)	0.55, 0.30-0.98
8.94+	30 (21)	33 (25)	0.41, 0.15-1.13
P for trend			*0.05*
IE63[e]			
<7.07	54 (38)	33 (25)	ref
7.07-<7.50	38 (26)	33 (25)	0.54, 0.34-0.86
7.50-<8.08	30 (21)	32 (24)	0.44, 0.23-0.81
8.08+	21 (15)	33 (25)	0.26, 0.12-0.58
P for trend			*<.0001*

[a] adjusted for age, sex, clustered by plate

[b] Number of participants in category and (percent)

[c] adjusted for ≤ 2 allergies vs. >2 allergies, age, sex clustered by plate

[d] adjusted for smoking (never, current, former), age and sex, clustered by plate

[e] adjusted for ≤ 2 allergies vs. >2 allergies, smoking, age and sex clustered by plate

Table 4A

Odds ratios and 95% confidence intervals for quartiles of log transformed VZV analyte levels associated with glioma stratified by sex, San Francisco Bay Area Adult Glioma Study.

VZV analyte quartiles	Sex				
	Women		Men		
gK					
	ctrl/ca	**OR 95% CI**	**ctrl/ca**	**OR 95% CI**	***pintx*[a]**
0	16/27	ref	17/29	ref	
1	9/16	1.05, 0.57-1.93	24/7	0.17, 0.05-0.55	
2	15/7	0.28, 0.12-0.65	16/18	0.66, 0.29-1.51	
3	12/8	0.40, 0.16-0.99	22/31	0.85, 0.44-1.64	
P for trend		0.006		0.72	*0.04*
gH					
0	18/27	ref	14/31	ref	
1	13/9	0.46, 0.33-0.64	21/18	0.38, 0.16-0.91	
2	6/19	2.11, 0.60-7.52	26/27	0.46, 0.25-0.83	
3	15/3	0.13, 0.04-0.44	18/9	0.22, 0.08-0.56	
		0.01		0.0009	*0.42*
gI					
0	14/20	ref	19/19	ref	
1	12/22	1.28, 0.66-2.49	20/21	1.0, 0.71-1.55	
2	12/8	0.46, 0.18-1.22	21/24	1.14, 0.76-1.69	
3	14/8	0.40, 0.17-0.93	19/21	1.09, 0.42-2.83	
		0.02		0.80	*<0.0001*
gE					
0	12/24	ref	19/27	ref	
1	15/11	0.36, 0.16-0.83	19/20	0.74, 0.46-1.22	
2	15/12	0.40, 0.23-0.69	18/14	0.55, 0.24-1.24	
3	10/11	0.55, 0.19-1.57	23/24	0.74, 0.58-0.94	
		0.15		<.0001	*0.44*
ORF2p					
0	14/25	ref	19/34	ref	
1	13/14	0.59, 0.32-1.10	20/20	0.56, 0.35-0.89	
2	10/10	0.56, 0.26-1.18	22/9	0.23, 0.14-0.36	
3	15/9	0.33, 0.17-0.64	18/22	0.67, 0.32-1.43	
		0.0001		0.04	*0.25*
ORF12p					
0	17/19	ref	16/26	ref	
1	12/16	1.17, 0.29-4.7	20/26	0.80, 0.38-1.67	
2	12/10	0.73, 0.35-1.52	22/19	0.52, 0.27-1.01	
3	11/13	1.04, 0.27-4.01	21/14	0.39, 0.20-0.75	
		0.86		0.001	*0.01*

VZV analyte quartiles	Sex				
	Women		Men		
ORF20p					
0	17/25	ref	16/30	ref	
1	11/12	0.74, 0.37-1.48	21/20	0.49, 0.20-1.20	
2	10/11	0.74, 0.29-1.89	23/17	0.38, 0.18-0.84	
3	14/10	0.48, 0.29-0.82	19/18	0.49, 0.26-0.95	
		<.0001		0.001	*0.73*
ORF24p					
0	13/19	ref	20/29	ref	
1	13/12	0.63, 0.26-1.51	20/17	0.59, 0.25-1.39	
2	13/15	0.79, 0.36-1.72	19/16	0.57, 0.35-0.93	
3	13/12	0.63, 0.25-1.58	20/23	0.79, 0.30-2.05	
		0.49		0.64	*0.86*
ORF26p					
0	11/20	ref	22/33	ref	
1	15/18	0.47, 0.17-1.30	17/17	0.67, 0.22-2.06	
2	12/12	0.54, 0.34-0.86	22/18	0.55, 0.19-1.57	
3	14/13	0.51, 0.18-1.49	18/17	0.63, 0.23-1.69	
		0.21		0.27	*0.95*
IE63					
0	16/21	ref	17/33	ref	
1	15/12	0.61, 0.22-1.63	18/26	0.72, 0.33-1.58	
2	8/13	1.22, 0.67-2.22	24/17	0.35, 0.11-1.05	
3	13/12	0.69, 0.32-1.51	20/9	0.21, 0.08-0.53	
		0.58		0.0001	*0.003*

[a] P-value for the interaction between sex in the association between each VZV antigen and glioma case status.

Table 4B

Odds ratios and 95% confidence intervals for quartiles of log transformed VZV analyte levels associated with glioma stratified by median number of allergies, San Francisco Bay Area Adult Glioma Study.

VZV analyte quartiles	Number of Allergies (median split)				
	≤ 2 allergies		>2 allergies		
gK	**ctrl/ca**	**OR 95% CI**	**ctrl/ca**	**OR 95% CI**	***pintx*[a]**
0	19/35	ref	13/17	ref	
1	14/13	0.51, 0.31-0.84	19/5	0.21, 0.04-1.03	
2	15/17	0.35, 0.22-1.87	16/6	0.30, 0.08-1.08	
3	18/24	0.72, 0.35-1.48	16/12	0.60, 0.25-1.43	
		0.48		0.32	*0.74*
gH					
0	19/35	ref	12/21	ref	
1	15/15	0.54, 0.32-0.94	19/8	0.25, 0.14-0.44	
2	15/30	1.08, 0.57-2.05	17/10	0.33, 0.12-0.86	
3	17/9	0.28, 0.13-0.61	16/1	0.04, .003-0.41	
		0.01		<.0001	*0.006*
gI					
0	17/27	ref	15/9	ref	
1	13/24	1.14, 0.90-1.43	19/14	1.18, 0.44-3.17	
2	20/20	0.62, 0.34-1.14	13/9	1.27, 0.46-3.54	
3	16/18	0.69, 0.26-1.84	17/8	0.80, 0.24-2.71	
		0.27		0.71	*0.67*
gE					
0	19/26	ref	11/20	ref	
1	15/22	1.11, 0.58-2.11	19/9	0.26, 0.14-0.48	
2	17/17	0.74, 0.45-1.22	16/6	0.20, 0.05-0.84	
3	15/24	1.24, 0.61-2.50	18/5	0.15, 0.04-0.59	
		0.81		0.003	*0.02*
ORF2p					
0	22/35	ref	10/21	ref	
1	14/25	1.12, 0.52-2.37	19/7	0.18, 0.07-0.43	
2	15/11	0.47, 0.26-0.83	17/5	0.14, 0.06-0.33	
3	15/18	0.75, 0.34-1.66	18/7	0.19, 0.08-0.44	
		0.14		0.0001	*0.01*
ORF12p					
0	21/28	ref	11/14	ref	
1	14/25	1.32, 0.35-4.90	18/15	0.66, 0.42-1.06	
2	19/20	0.77, 0.23-2.56	15/7	0.38, 0.11-1.25	
3	12/16	0.98, 0.29-3.27	20/4	0.16, 0.05-0.53	
		0.76		<.0001	*0 007*
ORF20p					

VZV analyte quartiles	Number of Allergies (median split)				
	≤ 2 allergies		>2 allergies		
0	23/32	ref	9/21	ref	
1	16/22	0.97, 0.29-3.22	16/8	0.22, 0.08-0.60	
2	14/19	0.96, 0.46-1.99	19/5	0.11, 0.04-0.32	
3	13/16	0.88, 0.31-2.54	20/6	0.13, 0.09-0.18	
		0.79		<.0001	*<0.0001*
ORF24p					
0	24/28	ref	8/17	ref	
1	12/18	1.28, 0.58-2.82	21/8	0.17, 0.09-0.32	
2	14/21	1.27, 0.54-2.97	18/7	0.18, 0.07-0.49	
3	16/22	1.18, 0.43-3.20	17/8	0.22, 0.08-0.55	
		0.74		0.01	*0.005*
ORF26p					
0	17/32	ref	15/18	ref	
1	18/21	0.60, 0.28-1.33	14/7	0.41, 0.11-1.55	
2	13/17	0.69, 0.38-1.24	21/11	0.43, 0.16-1.15	
3	18/19	0.58, 0.20-1.69	14/4	0.24, 0.06-0.97	
		0.33		0.03	*0.24*
IE63					
0	21/29	ref	11/22	ref	
1	22/27	0.84, 0.56-1.27	11/9	0.41, 0.10-1.71	
2	15/21	0.98, 0.41-2.39	17/6	0.17, 0.05-0.60	
3	8/12	1.05, 0.46-2.40	25/3	0.06, 0.02-0.17	
		0.87		<.0001	*<0.0001*

[a] P-value for the interaction between number of allergies in the association between each VZV antigen and glioma case status.