Title

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Journal

Human Genetics, 131(9)

ISSN

0340-6717

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Publication Date

2012-09-01

DOI

10.1007/s00439-012-1187-x

Peer reviewed

Published in final edited form as:
Hum Genet. 2012 September ; 131(9): 1507–1517. doi:10.1007/s00439-012-1187-x.

Insight in glioma susceptibility through an analysis of 6p22.3, 12p13.33-12.1, 17q22-23.2 and 18q23 SNP genotypes in familial and non-familial glioma

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Abstract

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CONFLICT OF INTEREST
None declared.

Background—The risk of glioma has consistently been shown to be increased two-fold in relatives of patients with primary brain tumors (PBT). A recent genome-wide linkage study of glioma families provided evidence for a disease locus on 17q12-21.32, with the possibility of four additional risk loci at 6p22.3, 12p13.33-12.1, 17q22-23.2, and 18q23.

Methods—To identify the underlying genetic variants responsible for the linkage signals, we compared the genotype frequencies of 5,122 SNPs mapping to these five regions in 88 glioma cases with and 1,100 cases without a family history of PBT (discovery study). An additional series of 84 familial and 903 non-familial cases were used to replicate associations.

Results—In the discovery study, 12 SNPs showed significant associations with family history of PBT ($P < 0.001$). In the replication study, two of the 12 SNPs were confirmed: 12p13.33-12.1 *PRMT8* rs17780102 ($P = 0.031$) and 17q12-21.32 *SPOP* rs650461 ($P = 0.025$). In the combined analysis of discovery and replication studies, the strongest associations were attained at four SNPs: 12p13.33-12.1 *PRMT8* rs17780102 ($P = 0.0001$), *SOX5* rs7305773 ($P = 0.0001$) and *STKY1* rs2418087 ($P = 0.0003$), and 17q12-21.32 *SPOP* rs6504618 ($P = 0.0006$). Further, a significant gene-dosage effect was found for increased risk of family history of PBT with these four SNPs in the combined data set ($P_{trend} < 1.0 \times 10^{-8}$).

Conclusion—The results support the linkage finding that some loci in the 12p13.33-12.1 and 17q12-q21.32 may contribute to gliomagenesis and suggest potential target genes underscoring linkage signals.

Keywords

Association; Polymorphisms; Glioma; Family history of primary brain tumor; Linkage analysis

INTRODUCTION

Glioma is the most common malignant primary brain tumor (PBT) and despite improvements in medical management is typically associated with very poor prognosis (Wen and Kesari 2008). To date, with the exception of exposure to ionizing radiation, no environmental or lifestyle risk factors for glioma have been identified. Epidemiological case-control and cohort studies have, however consistently shown a twofold increased risk for glioma associated with a family history of PBT (Hemminki and Li 2003; Wrensch et al. 1997; Goldgar et al. 1994) and approximately 5% of glioma patients report a family history of PBT (Bondy et al. 2008). Direct evidence for inherited genetic susceptibility to glioma is provided by the substantive risks of glioma associated with a number of Mendelian inherited disorders including neurofibromatosis types 1 and 2, tuberous sclerosis, retinoblastoma, Li-Fraumeni and Turcot syndromes (Bondy et al. 2008; Kyritsis et al. 2010). These diseases are rare and collectively, however, only account for ~1% of gliomas (Bondy et al. 1991).

Outside the context of these rare syndromes it is likely that the genetic architecture of glioma susceptibility is complex, involving the co-inheritance of multiple risk variants, epistatic interactions and gene-environment interactions. Support for the role of common genetic susceptibility to glioma has come from recent genomewide association studies (GWAS) which have identified variants at six loci influence disease risk (Liu et al. 2010). While these variants contribute significantly to disease burden in the population, the risks conferred are modest and they only explain a small proportion of the familial glioma risk.

Recently, the GLIOGENE (an acronym for "glioma gene") International Consortium conducted a linkage scan of 75 US glioma cancer families using a high-density single-nucleotide polymorphisms (SNPs) array provided evidence for a disease locus for glioma on 17q12-21.32 and three regions of suggestive linkage on 6p22.3, 12p13.33-12.1, and 18q23

(Shete et al. 2011). Applying an age of onset model to the linkage analysis of 74 of these families there was evidence of an additional linkage peak in 17q22-23.2 for familial glioma (Unpublished data).

While these data provide support for Mendelian susceptibility to non-syndromic glioma, the regions of linkage are large/broad and encompass many biologically plausible candidate genes. To seek to refine the chromosomal regions of interest and identify underlying genetic variants underscoring the linkage signals at 6p22.3, 12p13.33-12.1, 17q12-21.32, 17q22-23.2 and 18q23 we have conducted a family-based association study comparing SNP genotypes in glioma cases with (n = 88) and without (n = 1,100) a family history of PBT. We validated findings using an additional series of 84 familial glioma cases and 903 sporadic glioma cases.

MATERIALS AND METHODS

Subjects in Discovery Phase

The cases studied were ascertained from 1,281 glioma patients consecutively diagnosed and treated at The University of Texas MD Anderson Cancer Center, Houston, Texas (Shete et al. 2009; Liu et al. 2009b) between 1992 and 2008. Details on a family history of PBT and demographic variables were obtained from each patient using a standardized questionnaire administered by trained interviewers. Information on PBT in a first-degree relative (parent, sibling, or child) or second-degree relative (grandparent, aunt, uncle, or grandchild) was collected. We excluded 23 patients with self-reported non-European ancestry and 70 patients without information of family history of PBT leaving 1,188 patients for analysis. An attempt was made to validate reports of cancer in family members wherever possible through reference to medical records. A 20-ml EDTA-venous blood specimen was obtained from all patients. The study was approved by The University of Texas MD Anderson Institutional Review Board, and written informed consent was obtained from each patient.

Subjects in the Replication Phase

To validate findings we studied 987 patients from the National Cancer Institute (NCI; n = 315), and the Swedish Glioma Collection (n = 672). Detailed description of the NCI series was previously described (Inskip et al. 2001). Briefly, the NCI study was conducted between June 1994 and August 1998 at Brigham and Women's Hospital in Boston, MA; St Joseph's Hospital and Medical Center in Phoenix, AZ; and Western Pennsylvania Hospital in Pittsburgh, PA. The Swedish Glioma Collection comprised 275 glioma cases ascertained as part of the INTERPHONE Study conducted between 2000 and 2002 in Sweden (Cardis et al. 2007), and 397 cases from Umea° University Hospital and Neurosurgery University Clinics in Sweden. Both the NCI and Swedish studies were reviewed and approved by the respective institutional review boards, and all patients provided written signed an informed consent upon enrollment.

Selection of the SNPs

We analysed SNPs previously generated from GWAS of glioma (Shete et al. 2009), which map to 6p22.3 (NCBI build 36: 22,383,745-23,359,338bps), 12p13.33-12.1 (2,152,566–25,405,864bps), 17q12-21.32 (33,196,340 to 45,510,424bps), 17q22-23.2 (52,171,390–57,548,343bps), and 18q23 (from 73,651,628 to 76,085,595bps) and were represented on Human 610-Quad Bead Chip (Illumina, San Diego, CA). A total of 8,724 SNPs map to these five regions. To avoid analysis of highly correlated SNPs, we used HAPLOVIEW software (http://www.broad.mit.edu/mpg) to prune the dataset by imposing an LD threshold of $r^2 < 0.8$ and a minor allele frequency > 0.1 (because of the small number of cases with a family

history of PBT), thereby generating a total of 5,122 SNPs for analysis (Supplementary Table 1).

Statistical Methods

Genotype frequencies in glioma cases with and without a family history of PBT were compared using the χ^2 test. Odds ratio (ORs) and 95% confidence interval (CIs) were calculated by unconditional logistic regression analysis with adjustment for age, sex and histology (glioblastoma [GBM], and others). Akaike's information criterion was used to determine the best genetic model for each SNP (Akaike 1974).

To evaluate the chance of obtaining a false-positive association in the dataset, we used the Bayesian false-discovery probability (BFDP) test (Wakefield 2007). For the analyses, we used two levels (moderate 0.01, and low 0.001) of prior probabilities and the suggested BFDP cutoff value of 0.8 (Wacholder et al. 2004; Wakefield 2007). The joint effect analysis was evaluated by adding up the number of adverse alleles of the significant SNPs identified from the main effects analysis. Adverse alleles were defined as the minor allele of the risk SNPs and the common allele of the protective SNPs.

Pairwise LD was examined using *Lewontin*'s standardized coefficient *D'* (Lewontin 1988). The HAPLO.STATS was used for the haplotype analysis (http://www.mayo.edu/hsr/Sfunc.html) (Schaid et al. 2002). This method, based on the generalized linear model framework, allows adjustment for confounding variables and provides both global and haplotype-specific tests. Empirical *P*-values, based on 10,000 simulations, were computed for the global score test and each of the haplotype-specific score tests. All *P* values reported are 2-sided.

RESULTS

Patient Characteristics

Of the 1,188 cases in the discovery phase, 88 (7.4%) had a first- and/or second-degree relative with PBT (Table 1). of these family histories could only be validated by reference to patient medical records in 28 cases. Although not significant, glioma cases with a family history of PBT tended to be younger at diagnosis (median ages 45 and 48 years respectively; $P = 0.34$). The majority (54%) cases were GBM.

In the replication series, of the 987 cases, 84 (8.5%) reported a family history of PBT. Thirty-five of the 84 cases had verified first or second degree relative/relatives with glioma. As with discovery series, the majority (53%) of cases were GBM.

Individual SNP Association Analysis

In the discovery study, of the 5,122 tagging SNPs analyzed, 12 SNPs were noteworthy (BFDP ≤ 0.8) at a moderate prior probability level of 0.01, and showed significant associations with family history of PBT (Table 2). The genotype distributions of these 12 SNPs in cases with and without family history of PBT are summarized in Table 2. At the very low BFDP prior probability level of 0.001, two of these 12 SNPs remained noteworthy: rs1530364 and rs2418087 which annotate *WNT9B* and *STYK1*genes respectively.

The strongest signal was seen in *WNT9B* rs1530364 at 17q12-21.32, which remained significant after Bonferroni correction ($P = 3.5 \times 10^{-6}$; *P* adjusted = 0.018) (Fig 1 B). The second and third strongest signals were shown at 12p13.33-12.1, for *SOX5* rs7305773 ($P = 2.5 \times 10^{-5}$), and *STYK1* rs2418087 ($P = 5.7 \times 10^{-5}$) (Fig 1 A). Logistic regression analyses revealed that in the recessive-effect model cases without family history of PBT who had the

wild type or heterozygote genotype, significant risk effects were associated with having both family history of PBT and the variant homozygote of rs1530364 (OR= 3.91, 95% CI: 2.24–6.80), rs7305773 (OR= 5.67, 95% CI: 2.40–13.38), rs2418087 (OR 2.55, 95% CI: 1.59–4.08).

To confirm our finding that the 12 SNPs are not associated with glioma risk directly but associate with family history of PBT in glioma patients, three case-control analyses were performed: (1) the 1188 glioma cases (include the 88 cases with and 1100 cases without family history of PBT) versus 2236 controls whose genotype data are public available from The Cancer Genetic Markers of Susceptibility (CGEMS); (2) the 1100 glioma cases without family history of PBT versus the 2236 CGEM controls; and (3) 88 glioma cases with family history of PBT versus the 2236 CGEM controls. Results showing that in data sets (1) and (2), none of the 12 SNPs were significant ($P > 0.05$); in data set (3), all the 12 SNPs were still significant, but less significant when compared with the *P*-values seen in the present study (data not shown).

Haplotype Block Analysis

Since the tagged SNP approach identified two more SNPs in each of the four regions (*i.e.,* 6p22, 12p13, 17q12-21, 17q22-23.2), we further examined the association between the haplotypes and risk of family history of PBT. Table 3 details the frequencies of the haplotypes and risk of glioma in patients with and without a family history of PBT. Through this analysis we identified the following risk haplotypes “GG” (OR= 1.96, 95% CI: 1.22–3.16) in 6p22, “ACAT” (OR= 2.68, 95% CI: 1.55–4.37) in 12p13, “TAG” (OR= 2.71, 95% CI: 1.42–4.57) in 17q12-21.32, and “TT” (OR= 1.53, 95% CI: 1.04–2.23) and “CG” (OR= 1.93, 95% CI: 1.08–3.43) in 17q22-23.2. Consistent with the individual SNP analyses, all of the risk haplotypes carried the risk variants of the SNPs that were individually associated with increased risk of family history of PBT. The results of a global score test also showed statistically significant differences in the haplotype profile between cases with and without family history of PBT for all four regions (Table 3).

Replication Results

To identify the 12 SNPs showing evidence of an association at BFDP prior probability of 0.01 in the discovery study, we conducted replication in an independent case-control series involving 84 cases with family history and 903 cases without family history of PBT. In this analysis we adjusted for age, sex and histology. Only two SNPs, *PRMT8* rs17780102 (OR 1.99, 95%CI 1.06–3.76; $P = 0.031$) at 12p13.33-12.1, and *SPOP* rs6504618 (OR 1.95, 95%CI 1.08–3.52; $P = 0.025$) at 17q12-21.32, showed significant association with family history of PBT under recessive and dominant models respectively (Table 4). The second strong signal seen in the discovery study, *SOX5* rs7305773 at 12p13.33-12.1, showed only a marginally significant association.

Combined Analysis

Under a fixed-effects model, four of the 12 SNPs were noteworthy (BFDP ≤ 0.8) at a prior probability level of 0.01 in the combined analysis (Table 4). Three of the four SNPs localized to 12p13.33-12.1: *PRMT8* rs17780102 (OR = 2.13, 95% CI: 1.41–3.21; $P = 0.0001$), *SOX5* rs7305773 (OR = 3.53, 95% CI: 1.66–7.32; $P = 0.0001$), and *STKY1* rs2418087 (OR= 1.88, 95% CI: 1.30–2.71; $P = 0.0003$). An additional promising association signal was shown at 17q12-21.32, *SPOP* rs6504618 (OR = 2.01, 95%CI: 1.32–3.08; $P = 0.0006$).

Joint Effect Analysis

We next assessed the dose effect of the four SNPs found to be noteworthy (BFDP ≤ 0.8) at a prior probability of 0.01 from the combined data set. We treated the minor allele of each of the four risk SNPs as risk alleles with cases having zero risk allele as the reference group. The risk of family history of PBT increased progressively as the number of risk genotypes increased ($P_{trend} < 1.0 \times 10^{-8}$; Table 5). Specifically, compared with the referent group, the groups with two to three risk genotypes showed an increased risk of family history of PBT (OR = 3.72, 95% CI: 2.28–6.01).

DISCUSSION

By comparing genotype differences between glioma cases with and without a family history of PBT, we have provided evidence that genetic variation in the two linkage regions at 12p13.33-12.1 and 17q12-21.32 influence glioma risk. In the discovery study, the strongest signals were shown at 17q12-21.32 *WNT9B* rs1530364, 12p13.33-12.1 *SOX5* rs7305773 and *STYK1* rs2418087. In the replication study, the strongest three signals were seen in 12p13.33-12.1 *PRMT8* rs17780102, 17q12-21.32 *SPOP* rs650461, and 12p13.33-12.1 *SOX5* rs7305773. Although the number of familial cases was relatively small, to our knowledge this is the largest study genetic family history study of glioma thus conducted.

Two important genes map to the 17q12-21.32 region: *WNT9B* and *SPOP. WNT9B* (MIM #602864) belongs to the WNT gene family and has been implicated in the oncogenesis of a wide variety of cancers and in several developmental processes (Miyoshi et al. 1998; Smolich et al. 1993; Kirikoshi et al. 2001; Fukuchi et al. 1998). High levels of *WNT9B* expression are seen in the developing brain, spinal cord, cranial ganglia, and olfactory epithelium (Qian et al. 2003). Growing evidence implicates WNT pathway in important processes involved with the central nervous system (CNS) (Inestrosa and Arenas). Interestingly, the WNT pathway is appears to be involved in carcinogenesis of medulloblastoma (Zurawel et al. 1998; Dahmen et al. 2001). *SPOP* (speckle-type POZ domain protein, MIM # 602650) play a key role in early CNS and tumorigenesis (Dahmane et al. 2001). Furthermore, it was recently demonstrated that Knockdown of Drosophila *SPOP* mRNA expression by RNA interference (RNAi) and P-element insertion mutagenesis of the *SPOP* resulted in severe and consistent disruption of the peripheral and the CNS (Liu et al. 2009a).

The potential three target genes underscoring the 12p13.33-12.1 association were *PRMT8, STYK1* and *SOX5. PRMT8* (protein arginine methyltransferase 8, MIM # 610086) is specifically expressed in the brain and play an important role in neuronal differentiation and mediation of a nerve growth factor signal (Lee et al. 2005). *STYK1* (Serine/Threonine/ Tyrosine Kinase 1, MIM # 611433) play important roles in diverse cellular and developmental processes (Liu et al. 2004). Both overexpression and mutation data of *STYK1* have suggesting an oncogenic role for *STYK1* (Moriai et al. 2006; Jackson et al. 2009; Kondoh et al. 2009). *SOX5* (SRY-related high-mobility-group box 5, MIM # 604975) is a member of the high-mobility-group superfamily of transcription factors involved in the regulation of embryonic development. *SOX5* can suppress the oncogenic effects of platelet-derived growth factor B (PDGFB) signaling during glioma development, and was identified as a brain tumor locus in a retroviral insertional mutagenesis screen of PDGFB induced mouse gliomas (Tchougounova et al. 2009).

This study has provided evidence of an association between genetic variation at a number of regions and family history of PBT. Limitations of the study are that most associations seen in the discovery series could not be validated in the replication series, although they reached significant level in the combined analysis. However, replication failure should not be

interpreted as necessarily refuting the initial findings because of problems such as population stratification, genetic heterogeneity and restricted power. The discovery study and the NCI study cases are from US while the Swedish study participants were from Sweden. Furthermore, because the small number of glioma cases with family history of PBT, we could not accurately conduct any stratified analysis in different histology group. Secondly, this analysis is based on a case-only study design. We proposed this design as an acceptable alternative approach to the traditional approaches of case-control or cohort studies, given the historically low response rates among controls and that the disease is rare.

In conclusion, our findings support the genomewide linkage results pointing towards disease loci on 17q12-21.32 and 12p13.33-12.1 for glioma risk in families. Furthermore, our mapping efforts have identified plausible candidate genes for further study, namely, *WNT9B, SPOP, PRMT8, STYK1* and *SOX5*. The identification of the causal variants of these genes is likely to clarify the molecular mechanisms underlying gliomagenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health (5R01 CA119215, 5R01 CA070917 and R01CA52689). Additional support was provided by the American Brain Tumor Association, The National Brain Tumor Society, the Tug McGraw Foundation and the Wellcome Trust who provided funding for the parent GWAS.

The authors acknowledge the input of the Gliogene External Advisory Committee. For more information about the Gliogene Consortium, please refer to the following Web site http://www.gliogene.org.

ABBREVIATIONS

PBT	Primary brain tumor
SNP	single nucleotide polymorphism
GWA	genomewide association
LD	linkage disequilibrium
LOD	logarithm (base 10) of odds
OR	odds ratio
CI	95% confidence interval

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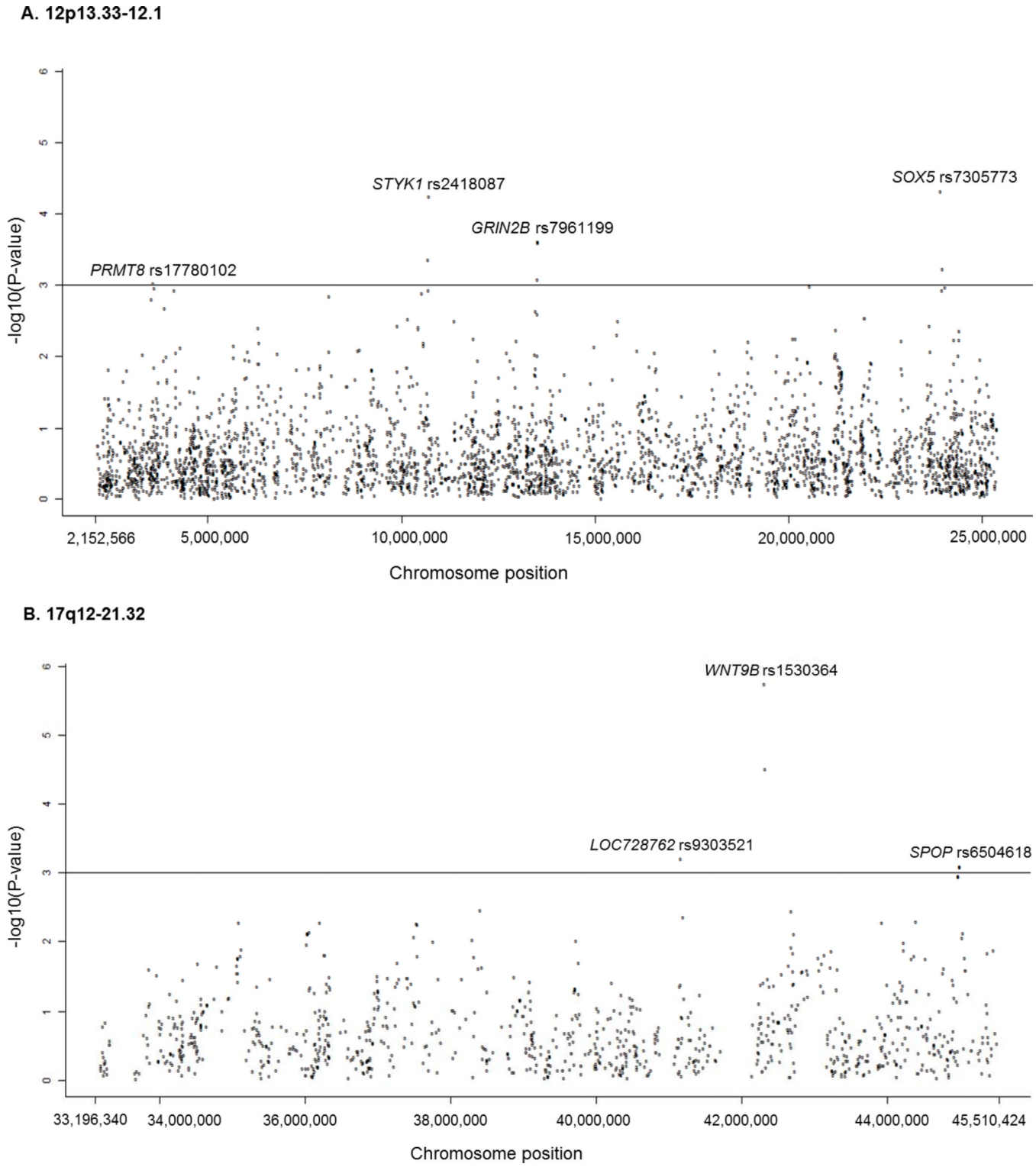

Figure 1. SNP distribution and association results for the two confirmed associated regions: 12p13.33-12.1 (A) and 17q12-q21.32 (B)

Chromosomal positions (x axis) based on NCBI build 36 coordinates. *P* values (as −log10 values; left y axis) are shown for SNPs analyzed in the discovery study. The solid dot means dominant model, the open dot means recessive model.

Table 1

Characteristics of glioma cases by family history of PBT

	Discovery study			Replication study		
	Family history of PBT, n. (%) *			Family history of PBT, n. (%) *		
Variable	**Yes (n=88)**	**No (n=1100)**	***P* value #**	**Yes (n=84)**	**No (n=903)**	***P* value #**
Age, years						
Median (range)	45 (20–70)	48 (18–86)	0.34 &	53 (26–81)	54 (17–89)	0.99
18–40	34 (38.6)	372 (33.8)		18 (21.4)	215 (23.8)	
41–60	42 (47.7)	553 (50.3)	0.63	38 (45.3)	397 (44.0)	0.89
≥ 61	12 (13.6)	175 (15.9)		28 (33.3)	291 (32.0)	
Sex			0.82			0.65
Male	53 (60.2)	681 (61.9)		46 (54.8)	520 (57.6)	
Female	35 (39.8)	419 (38.1)		38 (45.3)	383 (42.4)	
Histology			0.53			0.94
GBM	44 (50.0)	595 (54.1)		45 (53.6)	480 (53.2)	
non-GBM	44 (50.0)	505 (45.9)		39 (46.4)	423 (46.8)	
Family history of PBT ‡						
No	----	1100 (100)		---	903 (100)	
1st degree	24 (27.3)	---		20 (23.8)	---	
2nd degree	61 (69.3)	---		64 (76.2)	---	
1st and 2nd degree	3 (0.4)	---		0 (0)	---	

Note: PBT, primary brain tumor

*Numbers do not add up to the column totals due to missing values.

#By χ^2 test unless otherwise noted.

&By Wilcoxon rank sum test.

Table 2

Genetic variants showing strong association between glioma cases with and without family history of PBT ($P \leq 0.001$) in the discovery study

Chromosome	Gene	SNP	Genetic model *	Genotype	Family history of PBT, n. (%)		Logistic regression#		BDFP prior &	
					Yes (n=88)	No (n=1100)	OR (95% CI)	*P*-value	0.01	0.001
6p22.3	*PRL*	rs2064193	Dominant	AA	59 (67)	894 (81.3)	1 (reference)			
				GG+GA	29 (33)	206 (18.7)	2.15 (1.34–3.44)	7.0×10^{-4}	**0.77**	0.97
	LOC389370	rs16886628	Dominant	GG	70 (79.5)	1000 (91)	1 (reference)			
				TT+TG	18 (20.4)	99 (9)	2.65 (1.51–4.65)	5.0×10^{-4}	**0.69**	0.96
12p13.33-12.1	*PRMT8*	rs17780102	Recessive	GG+AG	67 (76.1)	976 (88.7)	1 (reference)			
				AA	21 (23.9)	124 (11.3)	2.42 (1.43–4.10)	0.001	**0.74**	0.97
	STYK1	rs2418087	Recessive	TT+CT	58 (65.9)	914 (83.1)	1 (reference)			
				CC	30 (34.1)	186 (16.9)	2.55 (1.59–4.08)	5.7×10^{-5}	**0.29**	**0.80**
	GRIN2B	rs7961199	Dominant	AA	65 (73.9)	602 (54.7)	1 (reference)			
				AG+GG	23 (26.1)	498 (45.3)	0.43 (0.26–0.70)	4.2×10^{-4}	**0.64**	0.95
	SOX5	rs7305773	Recessive	TT+CT	80 (90.9)	1079 (98.1)	1 (reference)			
				CC	8 (9.1)	21 (1.9)	5.67 (2.40–13.38)	2.5×10^{-5}	**0.55**	0.93
17q12-21.32	*LOC728762*	rs9303521	Recessive	GG+TG	57 (64.8)	885 (80.5)	1 (reference)			
				TT	31 (35.2)	215 (19.6)	2.26 (1.42–3.60)	4.7×10^{-4}	**0.63**	0.95
	WNT9B	rs1530364	Recessive	GG+AG	68 (77.3)	1018 (92.5)	1 (reference)			
				AA	20 (22.7)	82 (7.5)	3.91 (2.24–6.80)	3.5×10^{-6}	**0.02**	**0.17**
	SPOP	rs6504618	Dominant	AA	12 (13.6)	340 (30.9)	1 (reference)			
				AG+GG	76 (86.7)	760 (69.1)	2.85 (1.54–5.26)	4.5×10^{-4}	**0.74**	0.97
17q22-23.2	*LOC729430*	rs1879145	Dominant	CC	21 (24.7)	457 (42.4)	1 (reference)			
				TC+TT	64 (75.3)	621 (57.6)	2.22 (1.34–3.59)	0.001	**0.74**	0.97
	MSI2	rs868728	Recessive	TT+TG	81 (92.0)	1085 (98.6)	1 (reference)			
				GG	7 (8.0)	15 (1.4)	6.62 (2.60–16.62)	7.4×10^{-5}	**0.57**	0.93
18q23	*NFATC1*	rs7236492	Recessive	CC+TC	80 (90.9)	1076 (97.8)	1 (reference)			
				TT	8 (9.1)	24 (2.2)	4.61 (1.99–10.53)	1.7×10^{-4}	**0.71**	0.96

Note: PBT, primary brain tumor; OR, odds ratio; CI, confidence interval; BFDP, Bayesian false-discovery probability.

* Akaike's information criterion (AIC) was used to derive the best fitting genetic model for each SNP.

Adjusted for age, sex, and histology.

& Association at the 0.8 BFDP level are emboldened.

Table 3

Haplotype frequency between glioma cases with and without family history of PBT in the discovery study

Chromosome	Haplotype*	Family history of PBT, Haplotype frequency		OR (95% CI)#	*P*-value	Global score test &
		Yes (n=88)	No (n=1100)			
6p22.3	AG	0.76	0.87	1 (reference)		0.0010
	GG	0.13	0.08	1.96 (1.22 – 3.16)	0.005	
	Rare	0.11	0.05	2.24 (1.35 – 3.71)	0.002	
12p13	GTAT	0.20	0.25	1 (reference)		<0.0001
	GCAT	0.23	0.17	1.63 (0.91 – 2.90)	0.09	
	ATAT	0.11	0.12	0.99 (0.47 – 2.08)	0.98	
	ACAT	0.21	0.11	2.68 (1.55 – 4.37)	0.0003	
	Rare	0.25	0.35	1.61 (0.96 – 2.68)	0.07	
17q12-21.32	GGA	0.22	0.19	1 (reference)	---	0.00088
	TGA	0.19	0.17	1.11 (0.71 – 1.99)	0.88	
	GGG	0.19	0.15	0.83 (0.48 – 1.73)	0.49	
	TGG	0.17	0.14	1.17 (0.67 – 2.03)	0.73	
	TAG	0.08	0.16	2.71 (1.42 – 4.57)	0.003	
	rare	0.16	0.19	1.62 (0.87 – 3.09)	0.15	
17q22-23.2	CT	0.55	0.44	1 (reference)		0.027
	TT	0.30	0.35	1.53 (1.04 – 2.23)	0.03	
	CG	0.10	0.14	1.93 (1.08 – 3.43)	0.02	
	TG	0.05	0.07	1.68 (0.80 – 3.49)	0.17	

NOTE: PBT, primary brain tumor; OR, odds ratio; CI, confidence interval. Two loci chosen for 6p22: rs2064193 rs16886628; Four loci chosen for 12p13: rs17780102, rs2418087, rs7961199, rs7305773; Three loci chosen for 17q12-21: rs9303521, rs1530364, rs6504618; Two loci chosen for 17q22-23.2: rs1879145, rs868728.

*Haplotypes with a frequency <0.03 were pooled into a combined group.

#Adjusted for age, sex, and histology.

&From 10,000 simulations.

Table 4

Association results for the 12 SNPs in the replication and the combined studies

Chromosome	Gene	SNP	Genetic Model*	Replication study				Combined results			
				Family history of PBT, n. genotype counts #		OR (95%CI) &	*P*-value &	OR (95%CI)	*P*-value	BDFP	
				Yes (n=84)	No (n=903)					Prior 0.01	Prior 0.001
6p22.3	*PRL*	rs2064193	Dominant	67 | 17	710 | 192	0.85 (0.48–1.52)	0.59	1.47 (1.02–2.13)	0.03	0.98	0.99
	LOC389370	rs16886628	Dominant	79 | 5	824 | 77	0.77 (0.30–2.01)	0.60	1.60 (0.98–2.60)	0.04	0.98	0.99
12p13.33-12.1	*PRMT8*	rs17780102	Recessive	68 | 15	799 | 98	1.99 (1.06–3.76)	0.03	2.13 (1.41–3.21)	0.0001	**0.48**	0.90
	STYK1	rs2418087	Recessive	66 | 18	745 | 156	1.26 (0.71–2.25)	0.43	1.88 (1.30–2.71)	0.0003	**0.64**	0.95
	GRIN2B	rs7961199	Dominant	47 | 37	507 | 396	0.97 (0.61–1.55)	0.91	0.66 (0.47–0.93)	0.01	0.96	0.99
	SOX5	rs7305773	Recessive	81 | 3	883 | 17	2.99 (0.78–11.36)	0.09	3.53 (1.66–7.32)	0.0001	**0.77**	0.97
17q12-21.32	*LOC728762*	rs9303521	Recessive	69 | 15	705 | 196	0.85 (0.47–1.55)	0.60	1.41 (0.98–2.04)	0.05	0.97	0.99
	WNT9B	rs1530364	Recessive	80 | 4	839 | 62	0.58 (0.21–1.68)	0.31	2.09 (1.28–3.40)	0.0015	0.86	0.98
	SPOP	rs6504618	Dominant	15 | 69	256 | 645	1.95 (1.08–3.52)	0.02	2.01 (1.32–3.08)	0.0006	**0.76**	0.97
17q22-23.2	*LOC729430*	rs1879145	Dominant	34 | 50	338 | 565	0.87 (0.54–1.39)	0.55	1.38 (0.98–1.94)	0.05	0.98	0.99
	MSI2	rs868728	Recessive	82 | 2	880 | 23	0.79 (0.17–3.55)	0.76	2.86 (1.26–6.27)	0.004	0.95	0.99
18q23	*NFATC1*	rs7236492	Recessive	80 | 4	870 | 33	1.19 (0.4–3.56)	0.75	2.56 (1.27–5.04)	0.003	0.93	0.99

Note: PBT, primary brain tumor; OR, odds ratio; CI, confidence interval; BFDP, Bayesian false-discovery probability.

*Under a fixed-effects genetic model for each SNP according to the discovery data set.

#Genotype counts for Glioma cases with and without a family history of PBT.

&Adjusted for age, sex, and histology.

&Association at the 0.8 BFDP level are emboldened.

Table 5

Odds ratios corresponding to increasing numbers of risk variants of the four SNPs in the combined studies

No of risk variants *	Family history of PBT, n (%)		Logistic regression	
	Yes (n = 172)	No (n = 2003)	OR (95% CI)#	*P*-value &
0	21 (12.2)	420 (21.0)	1 (reference)	
1	78 (45.3)	1189 (59.4)	1.32 (0.81–2.12)	0.276
2	57 (33.1)	366 (18.3)	2.98 (1.79–4.90)	6.5×10^{-6}
3	16 (9.3)	28 (1.3)	11.41 (5.38–19.8)	1.0×10^{-7}
2~3	73 (42.4)	394 (19.6)	3.72 (2.28–6.01)	1.5×10^{-8}
Total	172 (100)	2003 (100)	*P* trend < 1.0×10^{-8}	

Note: PBT, Primary brain tumor; OR, odds ratio; CI, confidence interval.

*Risk genotypes were defined as the minor allele of the four SNPs as risk variants (*PRMT8* rs17780102, *STYK1* rs2418087, *SPOP* rs6504618 and *SOX5* rs7305773). We set cases with zero risk variants as the reference group, OR = 1.

#Adjusted for age, sex, and histology.

&*P* values for trend (two-sided) were derived from Extended Mantel-Haenszel chi square for linear trend tests (*df* = 1).