EDITORIAL

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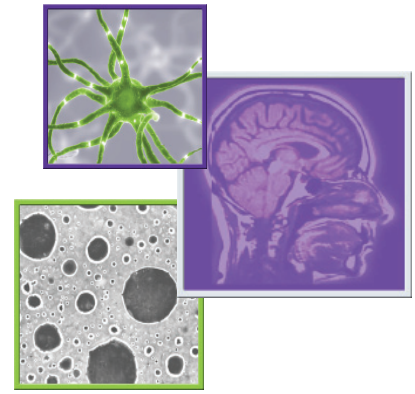
Rare and uncommon genetic variants may hold key to the 'missing heritability' in glioma



Kathleen M Egan*[1] Margaret R Wrensch[2] Robert B Jenkins[3]

"The extent to which rare and uncommon variants ... contribute to glioma remains to be determined. However, two recent studies provide the first evidence that this class of variants may contribute to 'missing heritability' in glioma..."

Glioma is the most common and lethal primary tumor of the brain with a dismal prognosis. While the etiology of these tumors is poorly understood, a hereditary component is suggested by the excess risk among relatives of glioma patients [1], and its occurrence in several inherited genetic syndromes including neurofibromatosis 1 and 2, tuberous sclerosis complex, von Hippel–Lindau disease, and Li–Fraumeni syndrome [2]. These diseases are extremely rare, however, and much of the excess familial risk is likely to be a consequence of multiple low-risk variants. Indeed, genome-wide association studies (GWASs) [3–6] have identified seven independent risk loci for glioma at 5p15.33 (*TERT*), 7p11.2 (*EGFR*; two independent loci), 8q24.21 (*CCDC26*), 9p21.3 (*CDKN2A/CDKN2B/CDKN2BAS*), 20q13.33 (*RTEL1*) and 11q23.3 (*PHLDB1*). Given that the arrays used in these GWASs were based on data from early haplotype maps and included only relatively common variants, all of the risk variants identified in these studies were of the common/low-penetrance type. The extent to which rare and uncommon variants (e.g., with a minor allele frequency <5%) contribute to glioma remains to be determined. However, two recent studies [7,8] provide the first evidence that this class of variants may contribute to 'missing heritability' [9] in glioma – one reporting the presumptive causal single nucleotide polymorphism (SNP) giving rise to the GWAS signal on chromosome 8 [7], and the other reporting a novel risk variant on chromosome 17 not previously implicated in GWASs [8]. Both of the novel variants identified in these studies were SNPs with minor allele frequencies of less than 5% and much higher relative risks than those found with common risk loci.

The chromosome 8 SNP was discovered in an investigation of the 8q24 loci first identified in the GWAS of Shete *et al.* [3]. Through a series of investigations involving

[1]Division of Population Sciences, Moffitt Cancer Center & Research Institute, Tampa, FL 33612, USA
[2]Department of Neurological Surgery & Institute of Human Genetics, University of California, San Francisco, CA 94143, USA
[3]Department of Laboratory Medicine & Pathology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA
*Author for correspondence: Tel.: +1 813 745 6149; Fax: +1 813 745 6525; kathleen.egan@moffitt.org

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imputation of SNPs around the tagged locus, next-generation sequencing, and validation of the top candidate SNPs by custom genotyping in two independent series of cases and controls, Jenkins *et al.* were able to narrow the search to a small region at the 8q24 locus with one SNP, rs55705857, producing the strongest signal [7]. When this SNP was included in models with other associated SNPs in the region, only the association of this SNP remained and the associations of the others became null, suggesting that rs55705857 is the cause of the association. The variant maps to an intron of *CCDC26* (ID: 137196), a gene not previously linked to any known gliomagenesis pathway. The region is highly conserved back to the platypus. While neither the function of the intronic region, nor of the G versus A allele, is known, the literature and modeling suggest that the region may encode for either a long noncoding RNA or microRNA.

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Of interest, Jenkins *et al.* found that the presumptively causal SNP at 8q24 was associated with oligodendroglial lineage tumors (pure oligodendrogliomas and mixed oligoastrocytomas), as well as astrocytic tumors of all grades that have acquired mutations in *IDH1/2* [7]. In contrast, the variant was minimally associated with astrocytic lineage tumors not bearing IDH mutations. *IDH1* and *IDH2* encode enzymes that convert isocitrate to α-ketoglutarate and are involved in maintaining the redox balance in the cell [10]. Oncogenic mutations at crucial arginine residues in these genes are thought to be an early initiating event in low-grade gliomas, causing a profound change in the pattern of DNA methylation in cells that carry the mutation [11,12]. *IDH1/2* mutations are present in more than 70% of low-grade gliomas and glioblastomas that evolved from clinically identified lower-grade tumors [13] (primary glioblastomas bearing these mutations are now thought to result from the progression of clinically undetected lower-grade tumors). *IDH1/2* mutations are also identified in a low proportion of acute or chronic myeloid neoplasms (5–10%), but are rare or absent in other tumor types. Interestingly, *CCDC26* is selectively expressed in the myelomonocytic lineage and is thought to play a role in myeloid cell differentiation and death [14], although the function of *CCDC26* in gliomagenesis is yet to be determined. Whilst the mechanistic link between *CCDC26* and IDH can only be speculated at this time, findings of Jenkins *et al.* show conclusively that the rs55705857 germline variant intronic to *CCDC26* predicts acquisition of IDH1/2 mutations in gliomas, one of the few such genotype/phenotype relationships yet established [7]. It is not yet clear whether the rs55705857 G allele actually facilitates the development of *IDH1/2* mutation or confers a selective advantage to cells that acquire such mutations. Nevertheless, these results also provide a proof of principle that 'rare variants' producing the signals in GWASs will be considerably more prominent than those produced by tagging SNPs [15]. In this regard, it is notable that the odds ratios (ORs) associated with rs55705857 G allele for all gliomas combined was markedly more elevated (per allele OR: ~3.1) than those reported for the SNPs tagging this locus (per allele ORs: 1.23–1.36) [3]. Strikingly, the excess risk associated with variant carrier status for this SNP in the report of Jenkins *et al.* is on the order of five to six for oligodendroglial lineage gliomas and *IDH1/2*-mutant astrocytic gliomas, the most prominent risk association reported yet for a SNP identified through GWAS and subsequently validated through sequencing [7]. The minor allele frequency among people with these tumor types was 21%, compared with 5% in controls and approximately 3% in the general Caucasian population. Thus, approximately 40% of patients with this subset of tumors carry at least one G (versus A) risk allele for rs55705857, compared with only approximately 8% of controls. Similarly, 3.5% of the patients carry two G copies compared with only 0.2% of controls.

The second low-frequency variant recently linked to glioma was initially identified in a study examining genetic susceptibility to cutaneous basal cell carcinoma (BCC) based on the Icelandic population [8]. In a GWAS of 16 million SNPs identified through whole-genome sequencing, the strongest signal for BCC risk genome-wide was produced by rs78378222 in the Icelandic population, and the same variant yielded significant results in other European populations. The SNP was not present in commercial arrays or in HapMap2 or HapMap3, and was discovered in the whole-genome sequencing phase of the study. The investigators subsequently examined whether this new BCC risk allele was associated with other cancer types by cross-referencing genotypes obtained using 'genealogy-based *in silico* genotyping' to nationwide Icelandic cancer databases. Through

this effort they observed significant associations of rs78378222 with a distinctive spectrum of tumors that included prostate cancer, colorectal adenoma and glioma. The association with glioma was subsequently validated in two separate US-based case–control samples, with a per allele OR of approximately 2.35 combining Icelandic and US data. A subsequent US-based case–control study [16] showed that the association could be demonstrated both in high-grade and low-grade tumors. Similar results have been observed by the University of California, San Francisco (CA, USA) and Mayo Clinic (MN, USA) groups [Unpublished Data]. The minor (C, at risk) allele is present at a frequency of approximately 0.015 in Caucasian populations, with some evidence that the carrier frequency declines with distance from northern Europe [8].

The rs78378222 variant resides in the 3′ untranslated region of *TP53,* a gene known to be mutated in a high proportion of diffuse gliomas [13]. The rs78378222 variant changes the AATAAA polyadenylation signal to AATACA, resulting in an impaired 3′-end processing of *TP53* mRNA. Thus, the variant is predicted to impact gene dose, but not transcriptional activity of the wild-type protein. Interestingly, Egan *et al.* reported a survival advantage in subjects carrying the variant allele [16], suggesting the possibility that it selects for tumors with a more indolent behavior, although this finding awaits replication (perhaps consistent with this interpretation, rs78378222 was associated with colorectal adenoma but not the more aggressive colorectal cancer in the report by Stacey *et al.* [8]). Glioma occurs in the spectrum of diseases associated with Li–Fraumeni and Li–Fraumeni-like syndromes that involve mutations in *TP53*. However, in the Icelandic population the rs78378222 variant was not associated with breast cancer, a common Li–Fraumeni syndrome tumor, suggesting, albeit indirectly, that this new variant does not account for rare occurrences of glioma in these families. It is possible that rs78378222 will be found to be associated with Li–Fraumeni-like cancer syndromes [17].

These two reports [7,8] should provide a catalyst for research focused on the detection and validation of other rare risk variants for glioma. All research in this area is challenged by the low incidence of these tumors and difficulties in accruing sufficient numbers of subjects for the study of rare exposures. Subtypes of glioma result from different molecular pathways and several of the GWAS variants are associated mainly with high-grade (*TERT* and *RTEL1*) or lower-grade (*CCDC26* and *PHLDB1*) tumors [18–20]. Given the low frequency of glioma, the detection of rare variants in strata of patients will necessitate the pooling of resources across studies. Such endeavors have recently paid off with the identification of the first two 'low-frequency–high-risk' variants in glioma, and acquisition of new information on the molecular pathways and mechanisms that give rise to these tumors.

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