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Survey of familial glioma and role of germline *p16^{INK4A}/p14^{ARF}* and *p53* mutation

Lindsay B. Robertson · Georgina N. Armstrong · Bianca D. Olver · Amy L. Lloyd · Sanjay Shete · Ching Lau · Elizabeth B. Claus · Jill Barnholtz-Sloan · Rose Lai · Dora Il'yasova · Joellen Schildkraut · Jonine L. Bernstein · Sara H. Olson · Robert B. Jenkins · Ping Yang · Amanda L. Rynerason · Margaret Wrensch · Lucie McCoy · John K. Wienkce · Bridget McCarthy · Faith Davis · Nicholas A. Vick · Christoffer Johansen · Hanne Bødtcher · Siegal Sadetzki · Revital Bar-Sade Bruchim · Galit Hirsh Yechezkel · Ulrika Andersson · Beatrice S. Melin · Melissa L. Bondy · Richard S. Houlston

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Abstract There is increasing recognition of familial propensity to glioma as a distinct clinical entity beyond a few rare syndromes; however its genetic basis is poorly understood. The role of *p16^{INK4A}/p14^{ARF}* and *p53* mutations in sporadic glioma provides a strong rationale for investigating germline mutations in these genes as a cause of familial glioma. To survey the familial glioma phenotype and examine the contribution of germline mutation in *p16^{INK4A}/p14^{ARF}* and *p53* to the disease we have analyzed a series of 101 index familial cases collected through the GLIOGENE Consortium (http://braintumor.epigenetic.org/). There was little evidence for within family correlations for tumour histology, suggesting generic susceptibility to glial tumors. We did not detect any functional mutations in *p16^{INK4A}* or *p14^{ARF}*. One index case with glioblastoma multiforme (GBM) diagnosed at age 54 and had a family history comprised of a paternal aunt with

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L. B. Robertson · B. D. Olver · A. L. Lloyd · R. S. Houlston (✉)
Section of Cancer Genetics, Institute of Cancer Research (ICR), Sutton, Surrey, UK
e-mail: richard.houlston@icr.ac.uk

G. N. Armstrong · S. Shete · M. L. Bondy
Department of Epidemiology, M.D. Anderson Cancer Center (MDACC), The University of Texas, Houston, TX 77030, USA

C. Lau
Baylor College of Medicine, Texas Children's Cancer Center (TCH), Houston, TX, USA

E. B. Claus
Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT, USA

E. B. Claus
Department of Neurosurgery, Brigham and Women's Hospital Boston (BW), Boston, MA, USA

J. Barnholtz-Sloan
Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH, USA

R. Lai
The Neurological Institute of Columbia University, New York, NY, USA

D. Il'yasova · J. Schildkraut
Cancer Control and Prevention Program, Department of Community and Family Medicine, Duke University Medical Center, Durham, NC, USA

J. L. Bernstein · S. H. Olson
Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Centre (MSK), New York, NY, USA

R. B. Jenkins · P. Yang · A. L. Rynerason
Mayo Comprehensive Clinic Cancer, Mayo Clinic, Rochester, MN, USA

M. Wrensch · L. McCoy · J. K. Wienkce
Department of Neurological Surgery, University of California, San Francisco (UCSF), San Francisco, CS, USA

B. McCarthy · F. Davis
Division of Epidemiology and Biostatistics, University of Illinois at Chicago (UIC), Chicago, IL, USA

GBM at age 55, carried the *p53* R158H mutation, which is predicted to be functional and has previously been implicated as a cause of Li-Fraumeni syndrome. Our findings provide no evidence that $p16^{INK4A}/p14^{ARF}$ and *p53* mutations contribute significantly to familial glioma.

Keywords *p16INK4A/p14ARF* · *p53* · Mutation · Familial glioma

Introduction

Gliomas are central nervous system neoplasms derived from glial cells and are comprised of astrocytomas, glioblastoma multiforme, oligodendrogliomas, and ependymomas. Gliomas account for ~80% of malignant primary brain tumors (PBT) and in the US ~21,000 individuals are diagnosed with glioma annually [1]. Most gliomas are unfortunately associated with a dismal prognosis despite surgery, radiotherapy and chemotherapy. While the only lifestyle exposure consistently linked to an increased risk of glioma is ionizing radiation, familial aggregation of the disease is well recognized with ~6% of patients having an affected relative. It is important to understand the basis of the glioma clustering in families for clinical management of glioma patients and their families (http://www.ncbi.nlm.nih.gov/sites/entrez).

In rare cases gliomas can arise in the context of single gene disorders, which include neurofibromatosis (MIM 162200), tuberous sclerosis (MIM 191100), Li-Fraumeni (MIM 151623) and Turcot's (MIM 276300) syndromes. These syndromes are, however, rare and collectively likely a priori to make a minor contribution to the 2-fold increased risk of glioma seen in first-degree relatives of patients [2].

The role of mutation in $p16^{INK4A}/p14^{ARF}$ and *p53* in sporadic glioma, coupled with the role of germline mutation as a basis of cancer predisposition, has provided a strong rationale for a number of researchers to evaluate the role of germline mutation in these genes as a basis for non-syndromic familial glioma [3–8]. Most of the studies published to date have been based on small sample sizes with limited power to robustly assess the contribution of mutations in these genes to familial glioma.

In 2004 at the Brain Tumor Epidemiology Consortium biannual meeting, we established GLIOGENE, an international consortium whose overarching goal is to ascertain and study glioma families [9]. GLIOGENE now involves multiple research groups in North America, Europe, and Israel and through this initiative a unique resource of glioma families has been established.

To survey familial glioma and provide a more complete examination of the contribution of germline mutation in $p16^{INK4A}/p14^{ARF}$ and *p53* to familial glioma we have analyzed a series of 101 index familial cases collected through GLIOGENE.

Methods

Patients

As gliomas are uncommon tumors in the general population we defined a family as having a predisposition to glioma if two or more individuals within the kindred had been diagnosed with a glial tumor. Under this definition we ascertained 101 families through the International GLIOGENE Consortium [9], which had not been the subject of previous molecular analyses. All 101 index patients had a histologically proven diagnosis of glioma and diagnosis of glioma in relatives has been established through reference to medical records or cancer registrations. Tables 1 and 2 summarize the number of affected individuals within each family, the age and diagnosis at presentation.

All biological samples were obtained from patients with informed consent and relevant local institutional review board (IRB) approval in accordance with the tenets of the declaration of Helsinki.

Mutational analysis

A search for mutations in the coding regions and splice sites of $p16^{INK4A}/p14^{ARF}$ and *p53* was performed by sequencing amplified PCR fragments using BigDye Terminator chemistry implemented on an ABI 3730xl

N. A. Vick
Department of Neurology, NorthShore University HealthSystem, Evanston, IL, USA

C. Johansen · H. Bødtcher
Institute of Cancer Epidemiology, Danish Cancer Society, Strandboulevarden, Copenhagen, Denmark

S. Sadetzki
Cancer and Radiation Epidemiology Unit, Gertner Institute, Chaim Sheba Medical Center, Tel Hashomer, Israel

R. B.-S. Bruchim
Department of Neurosurgery, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel

G. H. Yechezkel
Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

U. Andersson · B. S. Melin
Department of Radiation Sciences Oncology, Umeå University, Umeå, Sweden

Table 1 Summary of glioma families studied

Index case				Other family members		
Family ID	Origin	Sex/age	Pathology	Relation	Sex/age	Pathology
1	USA	F/48	O	Brother	M/33	A
				Pat. first cousin	F/20	E
2	USA	F/42	O	Brother	M/32	AA
3	USA	M/43	GBM	Sister	F/16	A
4	USA	F/40	A	Pat. first cousin	F/30	O
5	USA	M/51	GBM	Father	M/68	GBM
6	USA	M/40	AA	Brother	M/46	GBM
				Sister	F/16	A
				Brother	M/45	MEL
7	USA	F/54	GBM	Pat. uncle	M/57	GBM
				Pat. first cousin	M/51	GBM
8	USA	M/56	GBM	Sister	F/65	GBM
				Sister	F/53	GBM
9	USA	M/77	GBM, CRC	Pat. half-sister	F/68	GBM
				Pat. half-brother	M/66	AO
10	USA	F/42	GBM	Brother	M/42	A
11	USA	F/54	GBM	Pat. aunt	F/55	GBM
12	USA	F/27	AA	Brother	M/43	AO
				Sister	F/43	AA
13	USA	F/22	E	Mat. grand aunt	F/61	GBM
14	USA	F/27	O	Brother	M/39	AA
15	USA	F/52	GBM	Brother	M/61	GBM
16	USA	M/41	GBM	Pat. grandmother	F/52	A
17	USA	M/57	GBM	Brother	M/46	OA
				Sister	F/64	Men, MEL
18	USA	M/59	GBM	Mat. uncle	M/80	GBM
				Mat. first cousin, twice removed	M/60	GBM
19	USA	M/77	GBM	Nephew	M/40	AA
20	USA	F/34	OA	Mother	F/41	GBM
				Mat. first cousin, once removed	F/59	GBM
				Mat. aunt	F/45	G-U
21	USA	F/56	GBM	Nephew	M/13	GBM
22	USA	M/28	OA	Mother	F/60	GBM
23	USA	F/26	GBM	Father	M/45	GBM
24	USA	F/36	GBM	Mat. first cousin	M/17	O
25	USA	F/66	GBM	Father	M/70	GBM, LC
				Grandson	M/5	NE
26	USA	M/49	AA	Father	M/51	GBM
				Son	M/28	AO
27	USA	F/44	GBM	Mother	F/32	A
28	USA	F/51	A, GBM	Brother	M/41	AO
29	USA	F/42	GBM	Pat. grandfather	M/79	A
				Pat. first cousin, once removed	F/49	A
30	USA	F/19	A	Mother	F/33	O
31	USA	F/80	GBM	Brother	M/63	GBM
32	USA	F/36	PA	Son	M/11	A
33	USA	M/53	GBM	Sister	F/40	OA

Table 1 continued

Index case				Other family members		
Family ID	Origin	Sex/age	Pathology	Relation	Sex/age	Pathology
34	USA	M/65	AO	Mat. aunt	F/82	GBM
				Mat. first cousin, once removed	F/61	GBM
				Mat. grand aunt	F/81	AA
				Mat. grand aunt	F/79	GBM
				Mat. first cousin, once removed	F/63	G-U
35	USA	F/39	A	Mat. aunt	F/66	GBM
36	USA	M/38	A	Mat. grandfather	M/58	GBM
				Mat. grand aunt	F/75	GBM
37	USA	M/48	OA	Brother	M/40	O
38	USA	M/29	OA	Father	M/60	AO
39	USA	M/43	O	Brother	M/32	O
				Grand niece	F/7	PA
40	USA	F/45	OA	Mat. aunt	F/30	G-U
				Mat. grandmother	F/31	G-U
41	USA	M/64	A	Niece	F/32	A
42	USA	F/30	AA	Brother	M/12	E
43	USA	M/66	GBM	Brother	M/53	GBM
				Brother	M/54	LEU
44	USA	F/43	A	Mother	F/27	A
				Brother	M/45	GBM
45	USA	M/54	O	Sister	F/52	GBM
46	USA	F/74	A	Sister	F/82	GBM
				Mother	F/65	G-U
47	USA	F/16	PA	Mat. first cousin	M/6	PA
48	USA	M/68	GBM	Daughter	F/35	GBM
49	USA	F/5	G-U	Mat. aunt	F/10	AA
				Mat. first cousin, once removed	F/1	G-U
50	USA	F/6	A	Father	M/64	GBM
51	USA	M/28	AA	mz Twin	M/18	OA
52	USA	F/53	AO	Mat. first cousin	M/53	AA
53	USA	M/42	A, O	Brother	M/29	A
				Mat. uncle	M/38	A
54	USA	M/48	O	Father	M/55	OA
55	USA	F/68	GBM	Sister	F/60	A
				Brother	M/58	Men
56	USA	F/28	O	Mat. aunt	F/49	GBM
57	USA	F/51	O	Brother	M/38	GBM
				Brother	M/49	O
58	USA	F/53	GBM	Mat. uncle	M/58	GBM
59	USA	F/55	A	Sister	F/47	AA
60	USA	F/37	GBM	Sister	F/32	A
61	USA	F/73	GBM	Sister	F/45	OA
				Father	M/64	AA
62	USA	M/56	OA	Mother	F/83	GBM
63	USA	F/35	AE	Father	M/53	GBM
64	USA	M/68	GBM	Pat. first cousin	M/55	GBM
65	USA	M/66	GBM	Brother	M/71	GBM

Table 1 continued

Index case				Other family members		
Family ID	Origin	Sex/age	Pathology	Relation	Sex/age	Pathology
66	Denmark	F/45	A	Brother	M/37	GBM
67	Denmark	M/25	A	Father	M/39	O
				Pat. half aunt	F/60	GBM
68	Denmark	F/30	A	Mat. first cousin, once removed	F/37	GBM
				Mat. grand uncle	M/34	A
				Mat. grand uncle	M/75	GBM
69	Denmark	F/51	A	Brother	M/41	A
70	Denmark	F/35	GBM	Pat. grand aunt	F/50	GBM
71	Sweden	F/60	AE	Mother	F/70	A
				Brother	M/39	A
72	Sweden	F/46	AA	Nephew	M/9	A
73	Sweden	M/46	OA	Mat. uncle	M/51	GBM
74	Sweden	F/50	O	Pat. uncle	M/57	GBM
				Pat. first cousin	M/60	GBM
				Pat. first cousin	F/66	GBM
75	Sweden	F/54	GBM	Pat. uncle	M/48	GBM
76	Sweden	F/34	GBM	Mother	F/48	GBM
77	Denmark	M/58	AA	Father	M/40	GCG
78	Denmark	M/64	GBM	Father	M/61	O
				Son	M/2	ADR
79	Israel	M/0.5	ONG	Sister	F/16	GBM
80	Israel	F/36	A	Mother	F/40	A
81	Israel	F/47	O	Son	M/27	GBM
				Mother	F/59	MEL
82	Sweden	F/31	A	Father	M/63	GBM
83	Denmark	F/69	GBM	Brother	M/65	GBM
				Pat. uncle	M/67	GBM, PC
84	Denmark	F/56	O	Son	M/27	A
				Pat. first cousin	M/42	O, GBM
				Mat. first cousin	F/56	Men
85	Denmark	M/46	GBM	Pat. first cousin	M/58	AA
86	Denmark	F/29	A	Pat. first cousin	F/29	A
				Pat. great aunt	F/58	AO
				Pat. first cousin once removed	F/37	GBM
87	Denmark	M/80	GBM	Father	M/59	O
				Pat. second cousin	M/46	A
88	Denmark	F/67	AO	Brother	M/63	AO
89	Denmark	F/47	GBM	Father	M/68	GBM
				Mat. aunt	F/56	BC
				Mat. aunt	F/39	BC
				Mat. first cousin	M/30	SAR
90	Denmark	M/69	A	Mother	F/58	GBM
91	Denmark	M/77	GBM	Brother	M/69	GBM
				Mat. aunt	F/73	A
92	Denmark	F/53	GBM	Pat. first cousin	M/31	OA, AO
93	Denmark	M/43	AO	Mat. first cousin	M/39	GBM
94	Denmark	M/44	O	Mat. grandfather	M/74	AO

Table 1 continued

Index case				Other family members		
Family ID	Origin	Sex/age	Pathology	Relation	Sex/age	Pathology
95	Denmark	F/43	GBM	Pat. grandfather	M/58	GBM
96	Denmark	F/38	O	Father	M/46	GBM
97	Denmark	M/37	O	Father	M/68	AO
98	Israel	F/73	GBM	Sister	F/68	GBM
99	Israel	F/42	AA	Mat. second cousin	F/2	PA
100	Israel	M/58	GBM	Sister	F/58	GBM
				Brother	M/64	GBM
101	Israel	M/54	O	Mat. aunt	F/72	GBM

Age: age at diagnosis

Pathology: A, astrocytoma; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma: AE, anaplastic ependymoma; BC, breast cancer; CRC, colorectal cancer; GBM, glioblastoma multiforme; G-U, glioma unclassified; GCG, giant cell glioblastoma; E ependymoma; LEU, leukemia; LG, lung cancer; O, oligodendroglioma: OA, mixed oligoastrocytoma; ONG, optic nerve glioma; MEL, melanoma; Men, Meningioma; NE, neuroectodermal; PA, pilocytic astrocytoma; PC, pancreatic cancer; SAR, sarcoma

Table 2 Summary of the pathological classification of glioma in index patients and affected relatives

Glioma classification	WHO grade	Index case			Relative		
		N	Male	Median age (years)	N	Male	Median age (years)
Pilocytic astrocytoma (PA)	I	2	0	26	3	1 (33%)	6
Astrocytoma (A)	II	19	5 (26%)	40	25	12 (48%)	34
Oligodendroglioma (O)	II	15	6 (40%)	47	10	8 (80%)	40
Mixed oligoastrocytoma (OA)	II	7	5 (71%)	45	6	4 (67%)	43
Ependymoma (E)	II	1	0	22	2	1 (50%)	16
Giant cell glioblastoma (GCG)	II				1	1 (100%)	40
Anaplastic astrocytoma (AA)	III	8	4 (50%)	41	10	6 (60%)	45
Anaplastic oligodendroglioma (AO)	III	4	2 (50%)	59	9	8 (89%)	60
Anaplastic ependymoma (AE)	III	2	0	48			
Glioblastoma multiforme (GBM)	IV	41	18 (44%)	54	66	37 (56%)	58
Glioma, unclassified (G-U)		1	0	5	6	0	38
Optic nerve glioma (ONG)		1	1 (100%)	0.5			

sequencer (Applied Biosystems, CA, USA). PCR primers were designed using Primer 3 software to facilitate the investigation of all intron–exon boundaries (Supplementary Table 1). Sequence traces were aligned and compared to the gene consensus sequence using Mutation Surveyor (Version 3.0; SoftGenetics, PA, USA). Two in silico algorithms, PolyPhen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org) were used to predict the putative impact of missense variants on protein function. Scores were classified as tolerated, borderline or deleterious according to proposed criteria. Comparison of observed frequencies of sequence variants with those documented by dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) were performed using a test for the difference of two Binomial proportions as implemented in StatXact-7 (Cytel Studio, version 7.0.0).

Results

Thirty of the 101 kindreds (30%) had three or more family members affected by glioma. Only one of the families had six affected family members (Table 1). In three families melanoma had been diagnosed in three relatives (3%). In one family a relative with GBM was noted to have developed pancreatic cancer. In two of the families the constellation of cancer suggested the possibility of the Li-Fraumeni syndrome: In one family two relatives had

been diagnosed with breast cancer, at ages 56 and 39, and one relative diagnosed with sarcoma at age 30. In the other family the father had been diagnosed with an oligodendroglioma at age 61 years and the index patient's son diagnosed with adrenocortical carcinoma at 2 years of age (Table 1). In three families single relatives had been diagnosed with meningiomas (3%); these families had no distinguishing features.

In view of differences in the histological lineages of glial tumors we examined for concordance between pathologies in families; there was however little evidence for within family correlations suggesting generic susceptibility to glial tumors (Table 1).

Familial disease can be associated with earlier age of onset, indicative of genetic susceptibility. Ages at diagnosis of glioma in both index cases and relatives were similar to that observed in population based series [10] (Table 2). While a higher proportion of index cases were female, no significant sex differences were seen in affected relatives. Glioma tends to affect more males than females in the general population [1], hence the differences we observed may reflect ascertainment bias from the greater participation of females in our study rather than be reflective of sex-specific risk differences with respect to familial glioma.

We used direct sequencing to screen constitutional DNA from 101 glial patients from families in which two or more members had developed PBTs, most often GBM. Complete sequence data of the coding and splice site regions of $p16^{INK4A}/p14^{ARF}$ and *p53* were generated for all samples submitted for mutational analysis.

Mutational analysis of the coding regions identified eight sequence variants; four in $p16^{INK4A}/p14^{ARF}$ and four in *p53* (Table 3). Three of the four sequence changes in $p16^{INK4A}/p14^{ARF}$ have previously been documented by dbSNP as polymorphisms (rs37231249, rs11515, rs3088440). In addition we identified a novel synonymous change (19652C>T) in one patient (a male index case diagnosed at 46 years with mixed oligoastrocytoma with a maternal uncle diagnosed with GBM at 51).

Two of the four sequence changes identified in *p53* have previously been documented by dbSNP as polymorphisms (rs1042522, rs1800372) and genotype frequencies were not significantly different in cases from those previously documented in European populations (Table 3). One of the novel sequence changes identified was a synonymous change, thus predicted to have no deleterious consequences (11432T>C). The other sequence change identified was 12407G>A which leads to the substitution of arginine for histidine at amino acid 158 of the expressed protein (R158H). This mutation was identified in the index case of family 11, a non-Hispanic white individual and was diagnosed with a GBM at age 54. This individual's paternal aunt had been diagnosed with GBM at age 55 (Table 1). R158H is predicted by in silico algorithms to be deleterious and to have functional consequences on *p53*. This mutation is catalogued by the IARC *TP53* database (http://www-p53.iarc.fr/) as being causal of Li-Fraumeni syndrome. Intriguingly R158H has been proposed as being a low penetrance allele from analysis of childhood adrenocortical tumours [11].

Discussion

Evidence for a heritable basis to glioma is provided by the well documented elevated risk of glioma in relatives of patients. Furthermore, numerous anecdotal reports have described families in which multiple family members have developed glial tumors. Despite the assertion that multiple-case families can be a consequence of ascertainment, these families provide compelling evidence for genetic predisposition. The majority of these families have been nuclear

Table 3 Sequence variants identified in $p16^{INK4A}/p14^{ARF}$ and *p53*

Gene	Nucleotide change[a]	db SNP minor allele frequency[b]	Protein change	Minor allele frequency in the 101 cases (n)*
$P16^{INK4A}/P14^{ARF}$	19652C>T	–	Synonymous	0.005 (1)
	rs3731249: 23575G>A	0.017	A148T	0.030 (6)
	rs11515: 26292G>C	0.125	Synonymous	0.129 (26)
	rs3088440: 26332C>T	0.103	Synonymous	0.054 (11)
P53	rs1042522: 11392C>G	0.233	P72R	0.262 (53)
	11432T>C	–	Synonymous	0.005 (1)
	12407G>A	–	R158H	0.005 (1)
	rs1800372 12654A>G	0.016	Synonymous	0.025 (5)

* *P*-values for comparison of minor allele frequencies in cases with those obtained from dbSNP all >0.1

[a] Nucleotide position is taken from the GenBank reference file, dbSNP accession number shown where available

[b] In caucasians

clusters with inherited disease compatible with the full range of genetic models of predisposition. However, a number are large multi-generational families supporting the hypothesis of an autosomal dominant disease-causing allele with reduced penetrance as the basis of inherited susceptibility.

The molecular basis for the familial clustering of glioma is largely unknown. A small number of glioblastomas and astrocytomas arise in kindreds with inherited cancer syndromes that increase the risk of cancer at more than one site. For example, germ-line mutations in *p53* give rise to some cases of the Li-Fraumeni syndrome, which is characterized by the familial aggregation of breast cancer, sarcoma, childhood leukemia, and glioma. Similarly, germline mutations of the mismatch DNA repair genes *MLH1* and *PMS2* are implicated in Turcot syndrome in which patients develop gastrointestinal adenocarcinomas and glioblastomas. Finally, individuals with germ-line mutations of the *NF1* or *NF2* genes are prone to develop neurofibromatosis and occasionally develop gliomas as well. Collectively classical forms of these syndromes are very rare and thus genes associated with these syndromes make little contribution to the overall familial risk of glioma. This however is based on the premise that the classical forms of these diseases equates to mutational frequency.

Direct evidence for the role of $p16^{INK4A}/p14^{ARF}$ as a predisposition locus for both melanoma and glioma is provided by reports of single families which appear to define a melanoma-astrocytoma syndrome (MIM 155755); characterized by a dual predisposition to melanoma and neural system tumors, commonly astrocytoma [12, 13]. Support for an inter-relationship between the two tumor types is provided by the elevated risk of melanoma in relatives of glioma patients observed in some epidemiological studies [14, 15]. The underlying genetic basis for propensity to both tumor types in the families has been shown to involve germline deletions of 9p21, consistent with, loss of $p14^{ARF}$ function being the critical abnormality associated with this syndrome, rather than contiguous loss of $p16^{INK4A}/p14^{ARF}$ genes or disruption of expression or by mechanisms as yet unknown.

While the risk of melanoma associated with glioma is unlikely to solely reflect the melanoma-astrocytoma syndrome (MIM 155755) our analysis provides little evidence that archetypical $p16^{INK4A}/p14^{ARF}$ mutation plays a role in defining glioma susceptibility. This does not however, preclude the role of other forms of genetic variation influencing glioma risk. Evidence for this assertion comes from a genome-wide association study of glioma we have recently conducted which has shown that common single nucleotide polymorphisms (SNPs) mapping telomeric to $p16^{INK4A}$ confer a moderate but significant risk of glioma [16, 17]. While the population attributable fraction of glioma associated with these SNPs is high, the association translates to $\sim 1\%$ of the excess familial risk.

Knowledge of the molecular basis for familial aggregation of cancers is relevant to the clinical management of families both in terms of counseling and formulation of screening requirements. Here we have provided evidence that germline mutation in *p53* is unlikely to play a role in the development of familial glioma outside the context of Li-Fraumeni syndrome. We cannot exclude the possibility that a minority of mutations have been missed, or cannot be detected by a PCR-based approach, however our findings are in keeping with previously published smaller studies. Hence the role of germline mutations in $p16^{INK4A}/p14^{ARF}$ and *p53* are confined to the very small subset of syndromic glioma and this information should be integrated into clinical decision making processes.

The families such as the ones we have analyzed which exhibit the segregation of glioma compatible with Mendelian transmission provide a strong rationale for seeking to identify moderate-high penetrance mutations through genetic linkage. This is the overarching goal of GLIOGENE and through this initiative we have assembled a large family resource. Given that the power of linkage analysis to identify statistically significant linkage is limited by the existence of locus heterogeneity, the observation that germline $p16^{INK4A}/p14^{ARF}$ and *p53* mutations do not play a major role in familial glioma makes the prospects for identifying disease-loci through the linkage based strategy a better proposition.

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