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Progress Report Submitted to the National Brain Tumor Foundation

on

An Association Study of Adult Glioblastomas and DNA Repair Genes

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## **Introduction:**

The following is a progress report for *DNA Repair Genes and Glioblastoma* - a collaborative study of genetic variation in DNA repair pathways and risk of adult glioblastoma (GBM). A challenge of brain tumor epidemiology is the difficulty in assembling large numbers of brain tumor cases needed to study disease risk factors. A decision to combine data from several research institutions in the United States was made by investigators at the University of California at San Francisco (Drs. Margaret Wrensch and John Wiencke), the National Cancer Institute (Dr. Peter Inskip), the National Institute of Occupational Safety and Health (Drs. Avima Ruder and Mary Ann Butler), and MD Anderson at the University of Texas (Dr. Melissa Bondy) in order to improve power to study the causes of GBM. This project has allowed us to begin looking at the role of DNA repair genes on GBM risk and to establish procedures for future collaborative projects.

We hypothesize that genetic variation in DNA repair pathways could predispose adults to develop a GBM by influencing susceptibility to cellular damage that occurs as part of normal biological processes or susceptibility to environmental exposures.

**A. Specific Aims.** We propose to examine genetic differences in DNA repair pathway genes that may be associated with adult brain tumors by changing a person's susceptibility to endogenous (normal processes in the body) or exogenous (environmental) exposures. Specifically, we propose:

1. To study genetic differences in DNA repair pathway genes (including four different types of repair mechanisms: direct repair, base excision, nucleotide excision, and double strand break repair) and risk of adult glioblastoma.

As a secondary aim, we will examine the potential use of questionnaire data from the four study centers to explore how different types of occupational and environmental exposures combine with a person's genetic differences in DNA repair to influence risk of developing a glioblastoma.

## **B. Methods**

The current study is a collaborative analysis of genetic data from four population and hospital based case-control studies of glioma. Specifically, the study includes cases of adult glioblastoma (ICD-O code 9440) ages 18 and older that were confirmed through pathology review at each of the 4 study centers. The study is restricted to non-Hispanic white adults; a decision was made by investigators to restrict analysis to whites in the pilot, because only small numbers of individuals are available from other racial/ethnic groups. Both males and females are included. Genotyping is being completed for 11 DNA repair genes (14 variants) on 1,322 cases of GBM and 2,097 controls (Table 1). Because investigators at the 4 centers genotyped some of the DNA repair variants prior to initiating the collaborative study, the number of variants to be genotyped differs by study center (Table 2). Genotyping is in process at three laboratories: UCSF, MD Anderson at the University of Texas, and NCI (for both samples from NIOSH and NCI). To be certain that genotyping is consistent and valid across laboratories, 15 standard DNA samples with known genotypes were ordered from the Coriell Biorespository to be included in genotyping plates at each laboratory. Further, 5% of samples at each center are being genotyped twice to assess reliability of results. A TaqMan genotyping platform is being used at each of the 3 laboratories with primers and probes for each assay ordered from Applied Biosystems.

**Table 1. Genotyping list of DNA repair pathway genes for the NBTF GBM pilot study.**

	Gene	Acronym	Polymorphism	dbSNP ID	SNP500	TaqMan Assay Number
1	Methyl-guanine methyltransferase	<i>MGMT</i>	Leu84Phe	Rs12917	MGMT-06	001_0445 (V)
2	8-oxoguanine	<i>OGG1</i>	S326C	Rs1052133	OGG1-04	001_0694 (V)
3	Apurinic endonuclease 1	<i>APEX1</i>	Asp148Glu	Rs3136820	APEX1-03	002_0166 (V)
4	X-ray cross complementing gene 1	<i>XRCC1</i>	Arg194Trp	rs1799782	XRCC1-03	002_0211 (V)
5		<i>XRCC1</i>	Arg399Gln	rs25487	XRCC1-01	003_2127 (V)
6		<i>XRCC1</i>	Arg280His	rs25489	XRCC1-02	002_0210 (V)
7	ADP ribosyl transferase	<i>PARP</i>	Val762Ala	Rs1136410	PARP1-01	002_0161 (V)
8	Excision repair, cross-compl. 2 (or Xeroderma compl-D)	<i>ERCC2 (XPD)</i>	K751Q	Rs13181	ERCC2-03	001_0547 (V)
9	Excision repair, cross-compl, 5	<i>ERCC5</i>	His1104Asp	Rs17655	ERCC5-02	001_0688 (V)
10	DNA-dependent protein kinase	<i>XRCC7</i>	6721G>T		PRKDC	Assay By Design
11	RAD23B	<i>RAD23B</i>	Ala249Val	Rs1805329	RAD23B_04	001_0700 (V)
13	ERCC1	<i>ERCC1</i>	C8092A	rs3212986		001_0706 (V)
14	GLTSCR1	<i>GLTSCR1</i>	S397S	rs1035938		Assay By Design

**Table2. Genotyping of DNA repair variants by study center.**

	Gene	Polymorphism	dbSNP ID	NCI	NIOSH	MD Anderson	UCSF
1	<i>MGMT</i>	Leu84Phe	rs12917			Y	3,S
2	<i>OGG1</i>	S326C	rs1052133			Y	1,2,3,S
3	<i>APEX1</i>	Asp148Glu	rs3136820			Y	1,2,3,S
4	<i>XRCC1</i>	Arg194Trp	rs1799782			Y	3,S
5	<i>XRCC1</i>	Arg399Gln	rs25487				
6	<i>XRCC1</i>	Arg280His	rs25489				
7	<i>PARP</i>	Val762Ala	rs1136410			Y	1,2,3,S
8	<i>ERCC2 (XPD)</i>	K751Q	rs13181			Y	3,S
9	<i>ERCC5</i>	His1104Asp	rs17655			Y	1,2,3,S
10	<i>XRCC7</i>	6721G>T			Y	Y	1,2,3,S
11	<i>RAD23B</i>	Ala249Val	rs1805329	Y		Y	1,2,3,S
13	<i>ERCC1</i>	C8092A	rs3212986	Y	Y	Y	3,S
14	<i>GLTSCR1</i>	S397S	rs1035938	Y	Y	Y	1,2,3,S

Y=to be genotyped; 1=UCSF set 1; 2=UCSF set 2; 3= UCSF set 3; S= UCSF spore

### **C. Current Progress and Results**

Investigators selected the 14 DNA repair variants for genotyping and the case and control samples to be included in the pilot through several conference calls and following discussions at the 2005 Brain Tumor Consortium Meeting in Chicago. Laboratory work is in process at each of the 3 laboratories with the goal of completing genotyping for UCSF and MD Anderson in late August 2006. Genotyping work is being completed on 14 variants at MD Anderson, 11 variants at UCSF, and 3 variants for NCI and NIOSH samples. Coriell Biorepository standard samples (N=15) of known genotype (for quality control across laboratories) were selected to represent the possible genotypes for each DNA repair genotype (3 allele combinations each) and were shipped to each of the 3 laboratories.

Questionnaires from the 4 study centers were reviewed and summary tables of lifestyle and environmental variables potentially relevant to DNA repair genes and available from at least 3 of the 4 study centers were completed. Questionnaire data collected from the 4 centers include demographics, family history of cancer and brain cancer, head injury, history of therapeutic radiation, occupational history, and smoking history.

### **D. Summary**

Fourteen DNA repair gene variants were selected for study in this collaborative pilot project. The definition of eligible cases was defined as adult (ages 18 years or older) men or women diagnosed with a glioblastoma (ICD-O code 9440). Race/ethnicity was restricted to non-Hispanic white for purposes of the pilot, because too few cases in other racial/ethnic groups are available for independent analysis. Standard control samples were sent to each of the 3 laboratories to maintain quality control across study sites. Genotyping is in process at the 3 laboratories. Questionnaires from the 4 study centers were reviewed and tables describing questionnaire variables common to at least 3 of the centers and relevant to DNA repair genes were recorded. Questionnaire data and completed genotyping work (all but 3 of the variants) were sent by Dr. Ruder at NIOSH. Data from the remaining 3 centers will be assembled with the completion of the genotyping work. Analysis of genotyping data will be done following completion of the genotyping laboratory work.