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Maternal Variation in *EPHX1*, a Xenobiotic Metabolism Gene, Is Associated with Childhood Medulloblastoma: An Exploratory Case-Parent Triad Study

Philip J. Lupo, PhD[#1], **Darryl Nousome**[#2], **M. Fatih Okcu, MD, MPH**[1], **Murali Chintagumpala, MD**[1], and **Michael E. Scheurer, PhD, MPH**[1]

[1]Department of Pediatrics, Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX

[2]Human Genetics Center, Division of Epidemiology, Human Genetics and Environmental Sciences, University of Texas School of Public Health, Houston, TX

[#] These authors contributed equally to this work.

Abstract

Common epidemiologic study designs used for evaluating germline genetic determinants of childhood medulloblastoma are often subject to population stratification bias and do not account for maternal genetic effects, a proxy for the intrauterine environment, which may be important in determining etiologic factors for this outcome. The case-parent triad design overcomes these limitations. Therefore, we conducted an exploratory study among 27 childhood medulloblastoma case-parent triads recruited from the Childhood Cancer Epidemiology and Prevention Center at Texas Children's Hospital (Houston, TX) between 2003 and 2010. We assessed 13 single nucleotide polymorphisms (SNPs) in nine xenobiotic detoxification genes, as deficiencies in this pathway may induce brain tumorigenesis. Log-linear modeling was used to assess the association between medulloblastoma and both the offspring (i.e., case) and maternal genotypes of each SNP. In our population, there were no offspring genotypes that were significantly associated with disease risk. However, the maternal *EPHX1* rs1051740 genotype (RR=3.26, *P*=0.01) was associated with medulloblastoma risk. This exploratory study highlights the utility of the case-parent triad design, but these results should be interpreted cautiously due to the limited sample size.

Keywords

Case-parent triad; epidemiology; genetic polymorphisms; medulloblastoma; xenobiotic detoxification

INTRODUCTION

Central nervous system tumors account for about 27% of the cancer burden in childhood, of which medulloblastoma is the most common brain tumor [1]. Approximately 500 cases of medulloblastoma are diagnosed in the United States every year. The five-year survival rate

Correspondence to: Michael E. Scheurer, PhD, MPH, Baylor College of Medicine, One Baylor Plaza, MS-BCM305, Houston, TX 77030, 713-798-5547, scheurer@bcm.edu.

CONFLICT OF INTEREST STATEMENT
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of medulloblastoma is low (60%) [2], and therapy is associated with sequelae in neurocognition and physical deficits. There are few established risk factors for medulloblastoma, and its etiology is not well understood [3, 4]. The incidence of childhood medulloblastoma is bimodal with peaks at the ages of 3 to 4 years and 8 to 10 years [5], suggesting the importance of early life exposures, especially those that occur *in utero* [6].

It is hypothesized that there is an underlying genetic component to the development of childhood medulloblastoma [7]. Previous studies have examined the relationship between genetic polymorphisms in the xenobiotic detoxification pathway and medulloblastoma risk because of the role this pathway plays in modifying potential carcinogens [4, 8, 9]. Enzymes of the xenobiotic metabolism pathway are responsible for the elimination of exogenous and endogenous compounds through Phase I (e.g., oxidation) and Phase II (e.g., conjugation) reactions. If these compounds are “bioactivated” rather than eliminated, they may form DNA adducts, which can be carcinogenic. Because of the high rates of cell differentiation and proliferation during development, deficiencies in this pathway may induce brain tumorigenesis [3].

Limitations of epidemiological studies used to evaluate the genetic determinants of childhood medulloblastoma include small sample sizes, population stratification bias, and the inability to account for maternal genetic effects, which are a proxy for the intrauterine environment. Therefore, we conducted an exploratory case-parent triad study, using log-linear modeling, to evaluate the association between childhood medulloblastoma and 13 single nucleotide polymorphisms (SNPs) of nine genes of the xenobiotic detoxification pathway: cytochrome P450, family 1, subfamily A (*CYP1A1*); cytochrome P450, family 1, subfamily B (*CYP1B1*); cytochrome P450, family 2, subfamily E (*CYP2E1*); epoxide hydrolase 1 (*EPHX1*); glutathione S-transferase alpha 4 (*GSTA4*); glutathione S-transferase mu 3 (*GSTM3*); glutathione S-transferase mu 4 (*GSTM4*); glutathione S-transferase pi 1 (*GSTP1*); and N-acetyltransferase 2 (*NAT2*). The case-parent study design is robust to bias from population stratification in the estimation of offspring genetic effects, can be used to estimate the effects of both offspring (i.e., case) and maternal genotypes, and is generally more powerful than a traditional case-control study [10].

MATERIALS AND METHODS

Study Population

Medulloblastoma case-parent triads (n=27) were recruited from the Childhood Cancer Epidemiology and Prevention Center at Texas Children’s Cancer Center (Houston, TX) between 2003 and 2010. Patients with medulloblastoma were ≤14 years of age at diagnosis and the tumors were histopathologically confirmed (ICD-O-3 codes 9470-9474). Additionally, no restrictions were made on sex or race/ethnicity. After a written informed consent was obtained from the parent, we obtained DNA, blood or saliva, from each subject and parent. Participation of both parents was not required for our analysis [11]. These samples were used for genotyping. Demographic and clinical data were abstracted from medical records. The study protocol was approved by the Baylor College of Medicine Institutional Review Board.

SNP Selection and Genotyping

Nine genes of the xenobiotic detoxification pathway (CYP1A1, CYP1B1, CYP2E1, EPHX1, GSTA4, GSTM3, GSTM4, GSTP1, and *NAT2*) were selected because of their putative function or implicated role in tumorigenesis. Previous literature guided our selection strategies [8, 12]. SNPs with a minor allele frequency of <10% were not included.

With these criteria, 13 SNPs were selected for this analysis. Information on the genes on their corresponding SNPs and function is shown in Table 1.

We extracted DNA using the QIAmp DNA Blood Mini Kit (Qiagen, Valenica, CA) according to the manufacturer's protocol. Genotyping was performed using the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA) at the University of Texas School of Public Health according to the manufacturer's instructions.

Statistical Analysis

Characteristics of the subjects were summarized using counts and proportions. For each polymorphism, samples with genotyping failures; and for each subject, the number of genotyping failures was determined. Triads observed with genotype combinations inconsistent with Mendelian inheritance were also determined. These analyses were performed using Intercooled Stata, version 12.1 (StataCorp LP, College Station, TX).

Log-linear modeling was used to assess the association between medulloblastoma and both the offspring and maternal genotypes of each SNP [10]. Specifically, genotype relative risks (RR) and 95% confidence intervals (CI) were estimated using a log-additive model of inheritance. Therefore, the RR represents the increase or decrease in risk with each additional copy of the minor allele. A *P*-value for offspring and maternal genetic effects was determined using a likelihood ratio test (LRT) that compared the model that included terms for both offspring and maternal genotypes (i.e., full model), to models that included terms for only the offspring or only the maternal genotype (i.e., reduced models). These analyses were run using MI-GWAS with LEM [13, 14], which uses the expectation-maximization algorithm to allow the incomplete triads to contribute their information to the LRT without invalidation of the analysis [11]. Additionally, due to the number of comparisons, we controlled the false discovery rate (FDR) at 0.150 using the Benjamini and Hochberg method (*Q*-value) [15].

RESULTS

Genotyping was performed on samples from 27 families (64 individuals). Genotyping call rates ranged from 94% to 98%. A total of 13 SNPs were included in the analysis. Three individuals were excluded from further analysis based on genotyping failures for more than 6 SNPs (i.e., >50%). After these exclusions, the genotype call rates were >98% and considered of sufficient quality for analysis. No families were inconsistent with Mendelian inheritance, and therefore no exclusions were made based on this criterion. Of the families and individuals included for our analysis, 10 were complete triads, 14 were dyads, and 3 were monads.

The distribution of the characteristics of the medulloblastoma cases are presented in Table 2. The majority of the cases were male (70.3%). A majority of the population was non-Hispanic white (55.6%), followed by Hispanic (33.3%). Finally, a majority of the cases was under the age of 7 (63.0%). These numbers suggest that the population is representative of patients diagnosed in Texas.

Table 3 includes the estimates of the RR, 95% CI, *P*-values, and *Q*-values (i.e., FDR-adjusted *P*-values) for the association between the candidate maternal genotypes and childhood medulloblastoma. Although there were associations present between maternal genotypes and childhood medulloblastoma, most were not statistically significant. However, the maternal *EPHX1* rs1051740 SNP was significantly associated with medulloblastoma after adjusting for the FDR (RR=3.26, 95% CI: 1.12-9.53, *P*=0.010, *Q*=0.130). Specifically, the minor allele was associated with a threefold increase in the risk of medulloblastoma.

None of the offspring genotypes were significantly associated with disease risk in our population (Table 4). Two of the genotypes reached borderline statistical significance. The minor allele of *NAT2* rs1799930 was inversely associated with disease risk (RR=0.42, 95% CI: 0.17-1.06, *P*=0.055, *Q*=0.364), whereas the minor allele of *CYP1B1* rs1800440 was associated with medulloblastoma risk (RR=5.26, 95% CI: 0.65-42.60, *P*=0.056, *Q*=0.364).

DISCUSSION

Genetic variation in the xenobiotic detoxification pathway has been suggested as being associated with the risk of medulloblastoma. To our knowledge, this is the first study to examine the role of both offspring (i.e., case) and maternal xenobiotic metabolism genotypes in the context of childhood medulloblastoma risk. In this exploratory study, we conducted a case-parent triad analysis of 13 SNPs of nine genes in the xenobiotic metabolism pathway. While not conclusive, our results indicate the maternal *EPHX1* rs1051740 genotype may have a role in medulloblastoma etiology.

The epoxide hydrolases (e.g., EPHX1) are Phase I xenobiotic detoxification enzymes, which metabolize procarcinogens. *EPHX1* rs1051740 is responsible for a missense mutation resulting in a Tyr113His alteration in exon 3. This change is associated with 50% lower enzymatic activity of EPHX1 [16]. It is likely that the mutation produces enzymes that fail to detoxify carcinogenic epoxides which can lead to cellular DNA damage. This SNP has never been examined in relationship to medulloblastoma but has been associated with increased risk in childhood acute lymphoblastic leukemia [16]. Although our results were suggestive of a maternal association, there was no evidence of an association between the offspring *EPHX1* rs1051740 genotype and medulloblastoma risk.

As an exploratory study, our relatively small sample size may have limited our ability to detect modest associations between the genotypes and medulloblastoma. Due to this, caution should be taken when interpreting these results. Despite this limitation, our study provides evidence that the risk of medulloblastoma may be influenced by maternal xenobiotic detoxification. An important strength of this study was the use of the case-parent triad design, which allowed us to assess the effects of maternal genotypes [17].

In conclusion, we believe our results point to the usefulness of the case-parent triad study design in overcoming issues related to population stratification bias, the selection of appropriate controls, and limited sample size. This is especially important in the context of rare outcomes such as childhood medulloblastoma. Additionally, this study may inform future analyses of these pathways in the context of childhood medulloblastoma risk. The effect of the maternal *EPHX1* genotype may suggest that xenobiotic metabolism may be important *in utero* (i.e., maternal exposures during pregnancy). In the future, validation and replication of these results in a larger population and understanding the interaction between these genes and environmental factors will be necessary.

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ABBREVIATIONS

CI confidence interval

FDR	false discovery rate
LRT	likelihood ratio test
RR	relative risk
SNP	single nucleotide polymorphism

REFERENCES

1. Dhall G. Medulloblastoma. Journal of Child Neurology. 2009; 24:1418–1430. [PubMed: 19841429]
2. Ashley DM, Merchant TE, Strother D, Zhou T, Duffner P, Burger PC, Miller DC, Lyon N, Bonner MJ, Msall M, Buxton A, Geyer R, Kun LE, Coleman L, Pollack IF. Induction Chemotherapy and Conformal Radiation Therapy for Very Young Children With Nonmetastatic Medulloblastoma: Children's Oncology Group Study P9934. J Clin Oncol. 2012
3. Kunkle, B.; Sandberg, D.; Jayakar, P.; Felty, Q.; Roy, D. Gene–Environment Interaction and Susceptibility to Pediatric Brain Tumors. In: Roy, D.; Dorak, MT., editors. Environmental Factors, Genes, and the Development of Human Cancers. Springer; New York: 2010. p. 223-252.
4. Lupo PJ, Lee LJ, Okcu MF, Bondy ML, Scheurer ME. An exploratory case-only analysis of gene-hazardous air pollutant interactions and the risk of childhood medulloblastoma. Pediatric Blood & Cancer. 2012
5. Pizzo, PA.; Poplack, DG. Principles and Practice of Pediatric Oncology. Sixth ed.. Lippincott Williams & Wilkins; Philadelphia: 2011.
6. Coleman B, McLendon R, Kranz PG, Adamson DC. Medulloblastoma: Part I: Epidemiology, Clinical Presentation, and Histologic Characteristics. Contemporary Neurosurgery. 2012; 34:1–5.
7. Raffel C. Medulloblastoma: molecular genetics and animal models. Neoplasia. 2004; 6:310. [PubMed: 15256053]
8. Nielsen SS, Mueller BA, Preston-Martin S, Farin FM, Holly EA, McKean-Cowdin R. Childhood Brain Tumors and Maternal Cured Meat Consumption in Pregnancy: Differential Effect by Glutathione S-Transferases. Cancer Epidemiology Biomarkers & Prevention. 2011; 20:2413–2419.
9. Plon SE, Wheeler DA, Strong LC, Tomlinson GE, Pirics M, Meng Q, Cheung HC, Begin PR, Muzny DM, Lewis L. Identification of genetic susceptibility to childhood cancer through analysis of genes in parallel. Cancer genetics. 2011; 204:19–25. [PubMed: 21356188]
10. Weinberg CR, Wilcox AJ, Lie RT. A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. Am J Hum Genet. 1998; 62:969–78. [PubMed: 9529360]
11. Weinberg CR. Allowing for missing parents in genetic studies of case-parent triads. Am J Hum Genet. 1999; 64:1186–93. [PubMed: 10090904]
12. Barahmani N, Carpentieri S, Li XN, Wang T, Cao Y, Howe L, Kilburn L, Chintagumpala M, Lau C, Okcu MF. Glutathione S-transferase M1 and T1 polymorphisms may predict adverse effects after therapy in children with medulloblastoma. Neuro-oncology. 2009; 11:292–300. [PubMed: 18952980]
13. Agopian AJ, Mitchell LE. MI-GWAS: a SAS platform for the analysis of inherited and maternal genetic effects in genome-wide association studies using log-linear models. BMC bioinformatics. 2011; 12:117. [PubMed: 21513519]
14. Vermunt, JK. LEM: A general program for the analysis of categorical data. Tilberg University; 1997.
15. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological). 1995; 57:289–300.
16. Tumer TB, Sahin G, Arinc E. Association between polymorphisms of EPHX1 and XRCC1 genes and the risk of childhood acute lymphoblastic leukemia. Arch Toxicol. 2012; 86:431–9. [PubMed: 21983886]

17. Wilcox AJ, Weinberg CR, Lie RT. Distinguishing the effects of maternal and offspring genes through studies of “case-parent triads”. Am J Epidemiol. 1998; 148:893–901. [PubMed: 9801020]

Table 1

Xenobiotic detoxification genes and number of SNPs included in assessment of childhood medulloblastoma risk

Gene Name	Gene Symbol	Number of SNPs
Cytochrome P450, family 1, subfamily A	*CYP1A1*	1
Cytochrome P450, family 1, subfamily B	*CYP1B1*	2
Cytochrome P450, family 2, subfamily E	*CYP2E1*	1
Epoxide hydrolase 1	*EPHX1*	2
Glutathione *S*-transferase alpha 4	*GSTA4*	2
Glutathione *S*-transferase mu 3	*GSTM3*	1
Glutathione *S*-transferase mu 4	*GSTM4*	1
Glutathione *S*-transferase pi 1	*GSTP1*	1
N-acetyltransferase 2	*NAT2*	2

Table 2

Population characteristics of childhood medulloblastoma cases, Childhood Cancer Epidemiology and Prevention Center, 1987-2010

Case Characteristics		N (%)
Case Sex		
	Male	19 (70.3)
	Female	8 (29.7)
Race/Ethnicity		
	Non-Hispanic White	9 (33.3)
	Non-Hispanic Black	15 (55.6)
	Hispanic	1 (3.7)
	Other	2 (7.4)
Age at Diagnosis (years)		
	<3	7 (26.0)
	3-7	10 (37.0)
	>7-14	10 (37.0)

Table 3

Log-linear results for the association between maternal xenobiotic detoxification genotypes and childhood medulloblastoma

Gene	RefSNP	RR[1] (95% CI)	*P*-value[2]	*Q*-value[3]
EPHX1	rs1051740	3.26 (1.12, 9.53)	0.010	0.130
GSTA4	rs316133	1.59 (0.59, 4.26)	0.341	0.989
GSTM4	rs1010167	0.68 (0.27, 1.68)	0.390	0.989
CYP1B1	rs1056836	0.70 (0.26, 1.92)	0.480	0.989
EPHX1	rs2234922	1.65 (0.40, 6.80)	0.484	0.989
GSTP1	rs1695	1.96 (0.19, 20.72)	0.566	0.989
CYP2E1	rs2249695	0.77 (0.24, 2.50)	0.667	0.989
NAT2	rs1799930	1.16 (0.52, 2.57)	0.722	0.989
CYP1A1	rs4646903	0.92 (0.29, 2.90)	0.886	0.989
GSTA4	rs3756980	1.07 (0.39, 2.96)	0.891	0.989
NAT2	rs1799929	0.92 (0.25, 3.40)	0.901	0.989
GSTM3	rs1571858	0.97 (0.21, 4.47)	0.966	0.989
CYP1B1	rs1800440	0.99 (0.33, 2.96)	0.989	0.989

[1] RR represents the increase or decrease in risk with each additional copy of the minor allele

[2] Based on the likelihood ratio test comparing the model that included terms for both offspring and maternal genotypes, to reduced models that included terms for only the offspring or only the maternal genotype

[3] False discovery rate adjusted *P*-value

Table 4

Log-linear results for the association between offspring xenobiotic detoxification genotypes and childhood medulloblastoma

Gene	RefSNP	RR[1] (95% CI)	*P*-value[2]	*Q*-value[3]
NAT2	rs1799930	0.42 (0.17, 1.06)	0.055	0.364
CYP1B1	rs1800440	5.26 (0.65, 42.60)	0.056	0.364
GSTM3	rs1571858	0.49 (0.21, 1.15)	0.102	0.442
GSTP1	rs1695	1.67 (0.74, 3.78)	0.195	0.523
CYP1B1	rs1056836	0.53 (0.20, 1.40)	0.201	0.523
EPHX1	rs2234922	1.60 (0.57, 4.48)	0.354	0.722
GSTA4	rs3756980	0.62 (0.20, 1.91)	0.409	0.722
NAT2	rs1799929	1.47 (0.54, 3.99)	0.444	0.722
GSTA4	rs316133	0.77 (0.30, 1.97)	0.588	0.770
GSTM4	rs1010167	1.27 (0.46, 3.48)	0.642	0.770
CYP1A1	rs4646903	1.27 (0.45, 3.57)	0.651	0.770
EPHX1	rs1051740	1.18 (0.47, 2.93)	0.723	0.784
CYP2E1	rs2249695	1.02 (0.34, 3.06)	0.968	0.968

[1] RR represents the increase or decrease in risk with each additional copy of the minor allele

[2] Based on the likelihood ratio test comparing the model that included terms for both offspring and maternal genotypes, to reduced models that included terms for only the offspring or only the maternal genotype

[3] False discovery rate adjusted *P*-value