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Research Article

Childhood Brain Tumors and Maternal Cured Meat Consumption in Pregnancy: Differential Effect by Glutathione *S*-Transferases

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Abstract

Background: Some epidemiologic studies suggest that maternal consumption of cured meat during pregnancy may increase risk of brain tumors in offspring. We explored whether this possible association was modified by fetal genetic polymorphisms in genes coding for glutathione *S*-transferases (GSTs) that may inactivate nitroso compounds.

Methods: We assessed six GST variants: *GSTM1* null, *GSTT1* null, $GSTP1_{I105V}$ (rs1695), $GSTP1_{A114V}$ (rs1138272), *GSTM3**B (3-bp deletion), and $GSTM3_{A\text{-}63C}$ (rs1332018) within a population-based case-control study with data on maternal prenatal cured meat consumption (202 cases and 286 controls born in California or Washington, 1978–1990).

Results: Risk of childhood brain tumor increased with increasing cured meat intake by the mother during pregnancy among children without GSTT1 [OR = 1.29; 95% confidence interval (95% CI), 1.07–1.57 for each increase in the frequency of consumption per week] or with potentially reduced GSTM3 (any −63C allele; OR = 1.14; 95% CI, 1.03–1.26), whereas no increased risk was observed among those with GSTT1 or presumably normal GSTM3 levels (interaction $P = 0.01$ for each).

Conclusions: Fetal ability to deactivate nitrosoureas may modify the association between childhood brain tumors and maternal prenatal consumption of cured meats.

Impact: These results support the hypothesis that maternal avoidance during pregnancy of sources of some nitroso compounds or their precursors may reduce risk of brain tumors in some children. *Cancer Epidemiol Biomarkers Prev; 20(11); 2413–9. ©2011 AACR.*

Introduction

Childhood brain tumor (CBT) is the second most common pediatric cancer. Ionizing radiation is the only conclusively established nongenetic risk factor, but several epidemiologic studies suggest that maternal consumption of cured meats during pregnancy increases risk of CBT in offspring (1, 2). Although some studies have not observed this association (1, 3), the potential relationship remains compelling because cured meat is an important source of nitrite that can combine with other components of meat to form *N*-nitroso compounds (NOCs), including nitrosoureas (4). These are potent neurocarcinogens in non-human primates (5) and other animals, especially when exposure occurs *in utero* (6, 7).

Unlike some NOCs, nitrosoureas do not require enzymatic activation to act as carcinogens. Individual variation in a mother or child's ability to detoxify (denitrosate) these chemicals is the key to understand their potential impact on cancer risk. Glutathione *S*-transferases (GSTs) are important in the detoxification of nitrosoureas (8–10). These include the alpha (GSTA), mu (GSTM), pi (GSTP), and theta (GSTT) subfamilies. The various GSTs are structurally similar with some overlap in substrate specificity, but their activity with respect to nitrosoureas differs. The GSTs' relative expression levels in human brain, including during the fetal period, also differ. Therefore, some GSTs may play a more important role than others in protecting the fetal brain from nitrosourea compounds. Notably, GSTP1 is highly expressed in the fetal brain as early as 12 weeks gestation, including in astrocytes (11), the cell of origin for glial tumors, the tumor type most consistently associated with maternal cured meat consumption (2, 12). In addition, GSTP1 overexpression is associated with

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brain tumor resistance to the chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine) *in vitro* (13), consistent with a role of GSTP1 in nitrosourea metabolism in the brain. GSTT1 and GSTM3 are also highly expressed in the brain (14), and are particularly efficient in the metabolism of BCNU in humans (9). GSTA isoforms are not expressed in fetal brain (11). We thus focused our explorations on genetic polymorphisms in *GSTP1*, *GSTT1*, and the *GSTM4-GSTM2-GSTM1-GSTM5-GSTM3* gene cluster containing *GSTM3*.

Both *GSTM1* in this cluster and *GSTT1* contain a common genetic polymorphism that results in the complete absence of the respective enzyme activity among homozygous carriers of the variant allele (null status). The functional *GSTM1**A allele is linked with a 3-bp deletion (*B) in *GSTM3* that creates a Yin Yang 1 binding site (15). In the 5′ promoter resides another functional polymorphism, $GSTM3_{A\text{-}63C}$; the C allele is associated with reduced *GSTM3* expression (16). *GSTP1* contains two frequently studied polymorphisms, $GSTP1_{I105V}$ and $GSTP1_{A114V}$ that result in amino acid changes near the enzyme's catalytic center. These affect enzyme activity in a substrate-dependent manner, and are associated with survival among anaplastic glioma patients (17).

To elucidate the CBT–cured meat association, we examined whether it is modified by these 6 functional GST polymorphisms. Using population-based case–control data in which maternal cured meat consumption was associated with CBT (18), we assessed these polymorphisms by using DNA from dried blood spots (DBS) from newborn screening archives in California and Washington. We hypothesized that the previously observed CBT–cured meat association would be greater among children whose genotype might result in decreased denitrosation of nitrosoureas (i.e., reduced GST levels or activity) than among children with greater denitrosation capabilities.

Materials and Methods

Methods for obtaining interview data and specimens have been described (18–20). Briefly, all children were 10 years or younger and living in Seattle-Puget Sound (Washington), San Francisco-Oakland (California), or Los Angeles County (California) at the time of either diagnosis with a primary tumor of the brain, cranial nerves, or cranial meninges (ICD-O codes 191.0–192.1) in 1984–1991 ($N = 202$ cases) or recruitment via random digit dialing in 1989–1993 ($N = 286$ controls). These are all of the participants from the earlier population-based case–control study of CBT (18) for whom a DBS was located in state newborn screening archives. Among those born in California or Washington in a year with specimens archived (1978–1990), we obtained a DBS for 94% of cases and 86% of controls from Seattle (19), 93% of cases and 75% of controls from San Francisco, and 92% of cases and 85% of controls from Los Angeles (20). This represents 93% of cases and 83% of controls born in California or Washington in archived years, and 37% of cases and 36% of controls from the original study.

We ascertained frequency of maternal prenatal consumption of cured meat (ham, bacon, hot dogs, sausage, luncheon meat, and ''other cured meats'') by structured in-person interviews with mothers, on average 5.3 years after birth for cases and 6.4 years for controls. Institutional Review Board approvals were received from all relevant agencies prior to study initiation, informed consent was obtained prior to the interview, and DBS were anonymized prior to release from the archives. In Washington, specimens were labeled only with a randomly assigned identification number (19), and identifying information was removed from study data. Similar methods for assuring anonymity were used in California (20).

The Functional Genomics Laboratory at the University of Washington obtained DNA from DBS by using the QIAamp DNA Mini Kit (QIAGEN), and conducted genotyping for 6 variants: $GSTP1_{I105V}$ (rs1695), $GSTP1_{A114V}$ (rs1138272), *GSTM3**B (rs36120609-rs1799735-rs58210492), and $GSTM3_{A\text{-}63C}$ (rs1332018) by using TaqMan assays (Applied Biosystems); and *GSTM1* and *GSTT1* null by using one multiplex PCR-based assay (21). A portion of the β-globin gene was coamplified to verify that double-null status was not an artifact of PCR failure. Complete genotyping data were available for 200 (99%) cases and 279 (98%) controls. Duplicate or quadruplicate specimens for 6% of cases and 6% of controls from Washington were analyzed, blind to initial results, with complete concordance. When stratified by race/ethnicity, state, and case status, no genotype frequencies failed χ^2 tests for Hardy–Weinberg equilibrium, with the exception of Californian Hispanics for *GSTM3**B. However, this was statistically significant only for cases, and we confirmed that as reported previously (15) this allele was less frequent among GSTM1 null individuals ($P < 0.0005$, Pearson χ^2).

We estimated ORs and 95% confidence intervals (95% CI) for the CBT–cured meat association by using unconditional logistic regression, adjusted for study center, age, sex, and race/ethnicity. We categorized the latter as African American/black (either parent African American/black), Hispanic (either parent Hispanic, neither parent African American), white (both parents non-Hispanic white), and Asian/other. We adjusted for age, sex, and study center because they were frequency-matching variables, and for race/ethnicity because of previously reported associations with CBT, genotype, and cured meat consumption. Adjustment for birth year or maternal education did not materially affect ORs or CIs further, and therefore were not included in final models. For ORs between CBT and cured meat, we categorized maternal cured meat consumption as previously (18): never; ≤1 time/week; >1 time/week but ≤3 times/week; >3 times/week but ≤7 times/week; >7 times/week. We also evaluated consumption as a continuous (frequency per week) variable. We then stratified by genotype; dichotomization was required for *GSTT1* and *GSTM1* because the assay does not separate heterozygous and homozygous

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non-null individuals, and for the other 4 polymorphisms because homozygous variants were uncommon. We assessed interaction between maternal cured meat consumption (continuous) and genotype on the multiplicative scale, while including exposure and genetic main effects terms in the model. To the extent sample size allowed, we explored the consistency of results between our two largest racial/ethnic groups (non-Hispanic whites, non-black Hispanics); and by CBT histologic subtype [astroglial tumors (ICD-O histology codes 9380, 9382, 9400, 9401, 9420, 9421); medulloblastoma/primitive neuroectodermal tumors (PNET, codes 9470, 9471, 9473); and ''other'' tumors (all other codes)].

Results

Cases and controls for whom a DBS was located were similar to original study participants with regard to race/ethnicity and maternal education (Table 1). Those with DBS were born in more recent birth years (when archival samples were stored), and therefore were younger. The median age at diagnosis/reference for both cases and controls with DBS was 3 years (data not shown). Consistent with this relatively young age, proportionally fewer astroglial and proportionally more medulloblastoma/PNET cases were included than in the original study (Table 1). Only 3 (1%) cases and 3 (1%) controls had a personal or family history of Li–Fraumeni syndrome, neurofibromatosis, or tuberous sclerosis, or had a first-degree relative with a brain tumor (data not shown).

The CBT–cured meat association did not markedly vary by whether an archival DBS was obtained, although among the relatively contemporary group with DBS (median birth year, 1985), there was no indication of increased risk for the lowest category of exposure in slight contrast to those without a specimen (median birth year, 1977; Table 2). Similar to results reported for the full sample (18), the CBT–cured meat

Table 1. Demographic and clinical characteristics of children with and without brain tumors, overall and among those with a DBS for genotyping, West Coast Childhood Brain Tumor Study, births in 1965–1990

	All participants		Participants with a DBS for genotyping	
	Cases ($N = 540$)	Controls ($N = 801$)	Cases ($N = 202$)	Controls ($N = 286$)
Birth year				
1965–1977	194 (36)	292 (36)	–	–
1978–1984	232 (43)	325 (41)	99 (49)	142 (50)
1985–1990	114 (21)	184 (23)	103 (51)	144 (50)
Age at diagnosis/reference, y				
≤4	188 (35)	287 (36)	168 (83)	222 (78)
5–9	158 (29)	232 (29)	34 (17)	61 (21)
≥10	194 (36)	282 (35)	0 (0)	3 (1)
Study center				
Los Angeles	304 (56)	315 (39)	110 (54)	99 (35)
San Francisco	102 (19)	205 (26)	26 (13)	50 (17)
Seattle	134 (25)	281 (35)	66 (33)	137 (48)
Male	298 (55)	448 (56)	121 (60)	168 (59)
Race/ethnicity[a]				
Non-Hispanic white	313 (58)	532 (67)	105 (54)	192 (68)
Hispanic	147 (27)	183 (23)	62 (32)	61 (22)
African American	42 (8)	41 (5)	14 (7)	13 (5)
Asian/other	38 (7)	44 (6)	15 (8)	17 (6)
Maternal education (college)[a]				
None	270 (50)	318 (40)	103 (51)	112 (39)
Some	170 (32)	267 (33)	57 (28)	88 (31)
Degree	99 (18)	215 (27)	42 (21)	85 (30)
Histologic tumor type				
Astroglial	308 (57)	–	96 (48)	–
Medulloblastoma/PNET[b]	107 (20)	–	55 (27)	–
Other	125 (23)	–	50 (25)	–

NOTE: All values are given as *n* (%).

[a]Proportions exclude those with missing data on maternal race/ethnicity, paternal race/ethnicity, and/or maternal education.

[b]Primitive neuroectodermal tumor.

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Table 2. CBT and maternal consumption of cured meat during pregnancy, by availability of a DBS for genotyping, West Coast Childhood Brain Tumor Study, births in 1965–1990

Frequency of maternal cured meat[a] consumption during pregnancy (times/week)	Participants without a DBS for genotyping		Participants with a DBS for genotyping	
	Cases/controls ($N = 338/515$)[b]	OR (95% CI)[c]	Cases/controls ($N = 202/286$)[b]	OR (95% CI)[c]
Never	69/109	1.0 (reference)	35/51	1.0 (reference)
>0 to ≤1	66/97	1.26 (0.80–1.97)	38/73	0.84 (0.46–1.53)
>1 to ≤3	88/148	1.04 (0.69–1.58)	52/80	1.05 (0.59–1.86)
>3 to ≤7	76/112	1.29 (0.83–2.00)	54/63	1.37 (0.76–2.49)
>7	37/43	1.71 (0.91–3.05)	23/17	1.97 (0.88–4.41)
Continuous (per week)		1.04 (1.00–1.08)		1.03 (0.98–1.09)

Abbreviations: CBT, childhood brain tumor; DBS, dried blood spot.

[a]Ham, bacon, hot dogs, sausage, luncheon meat, or ``other'' cured meats combined.

[b]Tabulation excludes participants with missing cured meat data (2 cases and 6 controls without a DBS, and 2 controls with a DBS).

[c]Adjusted for age, study center, sex, and race/ethnicity (non-Hispanic white, Hispanic, African American, Asian/other).

association was suggested among participants with DBS but remained statistically nonsignificant for each of the 3 histologic tumor type categories (ORs of 1.68, 1.40, and 1.89 for cured meat >7 times/week vs. never for astroglial tumors, medulloblastoma/PNET, and ''other'' tumors, respectively; data not shown).

When we examined whether the CBT–cured meat association was modified by any of the selected functional polymorphisms, there was no indication that the CBT–cured meat association depended on either *GSTP1* polymorphism (Table 3). However, the association seemed modified by *GSTT1* genotype, with the association specifically observed among GSTT1 null children (Tables 3 and 4; interaction $P = 0.01$). We confirmed this interaction among the subset of non-Hispanic whites (interaction $P = 0.01$), but this subanalysis included only 12 GSTT1 null cases (data not shown). We also observed a statistically significant interaction with $GSTM3_{A\text{-}63C}$: The CBT–cured meat association was only present among children with the -63C (reduced expression) allele (Tables 3 and 5; interaction $P = 0.01$). When we explored whether this potential cured meat–$GSTM3_{A\text{-}63C}$ interaction varied by other polymorphisms in the same gene cluster, it remained, irrespective of *GSTM3**B (interaction $P = 0.04$–0.06) or *GSTM1* genotype (interaction $P = 0.03$–0.13; Table 3). In contrast, possible interactions between cured meat and *GSTM3**B and between cured meat and *GSTM1* disappeared when stratifying by $GSTM3_{A\text{-}63C}$ (also shown in Table 3).

We observed the *GSTT1*–cured meat interaction regardless of $GSTM3_{A\text{-}63C}$ genotype, and vice versa, although these interactions were not always statistically significant. The CBT–cured meat association was stronger among children with absent/reduced levels of both GSTT1 and GSTM3 (OR = 1.61; 95% CI, 1.17–2.22 for each increase per week in the frequency of consumption), than among those without GSTT1 but with normal *GSTM3* expression (OR = 1.10; 95% CI, 0.91–1.33), or those with reduced *GSTM3* expression but some GSTT1 (OR = 1.07; 95% CI, 0.96–1.19; Table 3). Risk of CBT did not increase with increasing exposure among children with both GSTT1 and normal *GSTM3* expression (OR = 0.95; 95% CI, 0.88–1.03). Although based on very sparse data, both the *GSTT1* and *GSTM3* interactions were suggested when we focused on astroglial tumors, medulloblastoma/PNET, or all other CBTs combined (all interaction $P \leq 0.11$; data not shown).

Discussion

To our knowledge, this is the first study to examine whether the previously observed CBT–cured meat association may be modified by the child's ability to metabolize potentially relevant carcinogens, as indicated by fetal *GSTT1*, *GSTP1*, *GSTM1*, and *GSTM3* genotype. For 2 of the 6 polymorphisms examined, any increase in CBT risk from prenatal cured meat was confined to children who presumably denitrosate (inactivate) NOCs more slowly, specifically those without GSTT1 (8), and carriers of the *GSTM3* -63C allele that is associated with reduced gene expression (16). These similar yet independent interactions between maternal prenatal cured meat intake and functional GST polymorphisms are biologically plausible. GSTT1 and GSTM3 are among the GSTs most highly expressed in the placenta and adult brain (14). In both organs, expression of GSTT1 and GSTM3 are at least an order of magnitude greater than GSTM1. Although GSTP1 is highly expressed in both placenta (14) and fetal brain (11), the well-studied *GSTP1* polymorphisms included here are amino acid changes that may not capture enzyme activity as well as a promoter region polymorphism such as $GSTM3_{A\text{-}63C}$, or the *GSTT1* null

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Table 3. CBT and maternal consumption of cured meat during pregnancy, by selected functional polymorphisms in genes coding for 4 GST enzymes, overall and by *GSTM3*$_{C\text{-}63A}$, West Coast Childhood Brain Tumor Study, births in 1978–1990

Genotype	All participants with DBS for genotyping		*GSTM3* -63AA (normal expression)		*GSTM3* -63AC/CC (reduced expression)	
	Cases/controls (N = 202/286)[a]	OR (95% CI)[b]	Cases/controls (N = 85/113)[a]	OR (95% CI)[b]	Cases/controls (N = 117/169)[a]	OR (95% CI)[b]
GSTP1$_{I105V}$						
VV/IV	117/191	1.03 (0.96–1.10)	45/79	0.95 (0.86–1.05)	72/109	1.23 (1.08–1.41)
II	85/94	1.04 (0.96–1.12)	40/34	1.03 (0.90–1.18)	45/59	1.03 (0.88–1.21)
GSTP1$_{A114V}$						
VV/AV	23/53	1.07 (0.95–1.20)	9/24	1.21 (0.93–1.56)[c]	14/27	1.35 (0.93–1.96)
AA	179/232	1.05 (0.98–1.11)	76/89	0.99 (0.91–1.08)	103/141	1.13 (1.01–1.26)
GSTT1						
Not null	169/235	1.00 (0.96–1.05)	72/97	0.95 (0.88–1.03)	97/136	1.07 (0.96–1.19)
Null	31/50	1.29 (1.07–1.57)	12/16	1.10 (0.91–1.33)	19/32	1.61 (1.17–2.22)
GSTM1						
Not null	105/140	1.06 (0.98–1.14)	41/54	1.00 (0.90–1.12)	64/83	1.13 (0.99–1.30)
Null	95/145	1.01 (0.93–1.08)	43/59	0.96 (0.87–1.06)	52/85	1.18 (1.01–1.38)
*GSTM3**B						
Any *B	68/94	1.00 (0.91–1.09)	43/52	0.98 (0.89–1.08)	25/39	1.24 (0.96–1.61)
No *B	134/191	1.06 (1.00–1.13)	42/61	0.98 (0.89–1.08)	92/129	1.15 (1.03–1.27)
GSTM3$_{A\text{-}63C}$						
AA	85/113	0.98 (0.91–1.05)	–	–	–	–
AC/CC	117/169	1.14 (1.03–1.26)	–	–	–	–

[a]Numbers may not add to total due to missing genotyping data.

[b]Per frequency of maternal prenatal consumption per week of cured meats (ham, bacon, hot dogs, sausage, luncheon meat, or ``other'' cured meats combined), adjusted for age (continuous), study center, sex, and race/ethnicity (non-Hispanic white, Hispanic, African American, Asian/other) unless noted, excludes 2 controls without cured meat data and ≤2 cases and ≤4 controls without genotyping data.

[c]Restricted to non-Hispanic whites (excludes 6 Hispanic controls) to control for race/ethnicity.

polymorphism resulting in a complete absence of enzyme activity. In addition, of the GSTs considered here, GSTT1 and GSTM3 may be the most efficient in inactivating nitrosoureas (9). Together, these results suggest that the possible association between cured meat consumption during pregnancy and CBT risk in offspring may be modified by the fetus' ability to metabolize compounds potentially associated with the consumption of cured meats, such as nitrosoureas (4).

Care must be taken in interpreting these results. First, our sample size was modest, which increased the probability of false positives (22). Second, the interactions were present in each histologic group, including the highly heterogeneous ''other'' tumors. This was unexpected because most epidemiologic studies suggest that the CBT–maternal cured meat association may be specific to astroglial tumors (2–3, 12, 23), as may be any association with nitrate or nitrite in tap water (24). However, in animal studies nitrosoureas induce a variety of brain tumor types (25). Also, the lack of tumor-specific associations does not suggest selection or information bias, because generally neither inflates interactions (26–27). Finally, much remains to be learned about the content of specific NOCs and nitrosatable alkylureas in cured meat or their *in vivo* formation (4); their detoxification by individual GSTs; and the expression of individual GSTs in fetal brain and placenta over the course of pregnancy. Animal models suggest species-specific periods of susceptibility. They also indicate that nitrosation inhibitors such as vitamin C prevent neurogenic tumors in offspring of rodents simultaneously exposed to nitrite and nitrosatable ureas during pregnancy (28). Therefore, it is a limitation that our modest sample size combined with a nearly universal use of vitamin supplements precluded examination of the observed interactions by supplement use. Despite these limitations, this work builds on earlier studies focused either on cured meat (1–3, 12, 23) or GST genetic (29–31) main effects. Our results underscore the importance of considering genotype when assessing CBT–exposure associations. They also may suggest the need to assess multiple GSTM functional polymorphisms in studies of CBT and perhaps other outcomes relevant to substrates better metabolized by GSTM3 than GSTM1. These genes both reside in the *GSTM4-GSTM2-GSTM1-GSTM5-GSTM3* gene cluster, and until stratifying by

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Table 4. CBT and maternal consumption of cured meat during pregnancy, by fetal *GSTT1* genotype, West Coast Childhood Brain Tumor Study, births in 1978–1990

	GSTT1 non-null (some GSTT1)		*GSTT1* null (no GSTT1)	
Cured meat[a] consumption during pregnancy (times/week)	Cases/controls ($N = 169/235$)[b]	OR (95% CI)[c]	Cases/controls ($N = 31/50$)[b]	OR (95% CI)[d]
Never	33/41	1.27 (0.66–2.46)	2/10	0.51 (0.04–3.53)
>0 to ≤1	31/55	1.0 (reference)	7/18	1.0 (reference)
>1 to ≤3	46/70	1.21 (0.67–2.19)	5/10	1.29 (0.25–6.23)
>3 to ≤7	44/51	1.46 (0.79–2.71)	9/11	3.64 (1.02–13.55)
>7	15/16	1.48 (0.61–3.58)	8/1	

[a]Frequency of consumption of ham, bacon, hot dogs, sausage, luncheon meat, or ``other'' cured meats combined.
[b]Tabulation excludes 2 controls with missing data on maternal cured meat consumption.
[c]Adjusted for race/ethnicity, study center, age, and sex.
[d]Exact unadjusted OR and 95% CI.

Table 5. CBT and maternal consumption of cured meat during pregnancy, by fetal $GSTM3_{A\text{-}63C}$ genotype, West Coast Childhood Brain Tumor Study, births in 1978–1990

	GSTM3 -63AA (normal expression)		*GSTM3* -63AC/CC (reduced expression)	
Cured meat[a] consumption during pregnancy (times/week)	Cases/controls ($N = 85/113$)	OR (95% CI)[b]	Cases/controls ($N = 117/169$)[c]	OR (95% CI)[b]
Never	16/24	1.0 (reference)	19/27	1.0 (reference)
>0 to ≤1	22/24	1.52 (0.62–3.73)	16/46	0.55 (0.23–1.31)
>1 to ≤3	18/32	0.86 (0.35–2.09)	34/48	1.22 (0.55–2.69)
>3 to ≤7	22/21	1.86 (0.74–4.71)	32/41	1.20 (0.53–2.69)
>7	7/12	0.73 (0.22–2.38)	16/5	5.66 (1.62–19.78)

[a]Frequency of consumption of ham, bacon, hot dogs, sausage, luncheon meat, or ``other'' cured meats combined.
[b]Adjusted for race/ethnicity, study center, age, and sex.
[c]Tabulation excludes 2 controls with missing data on maternal cured meat consumption.

$GSTM3_{A\text{-}63C}$, it unexpectedly seemed that the CBT–cured meat association was present among children with GSTM1 but not among GSTM1 null children. In addition, given some overlap in function, it may also be important to consider the joint effects of polymorphisms in different GST subfamilies, including *GSTM3*, *GSTT1*, and *GSTP1*. Our ability to do this in the context of estimating CBT–cured meat ORs was limited, and the corresponding results can only be viewed as exploratory.

This work supports the premise that some NOCs and NOC precursors may play a role in initiation of brain tumors during human fetal development. Future studies will benefit from assessment of maternal cured meat intake by trimester of pregnancy, larger sample sizes, and the inclusion of children conceived in a wider range of birth years to examine the effect of decreasing levels (4) of nitrite in cured meats over time. It also may be informative to genotype both mothers and children, so that the effect of GST enzymes in mothers' livers can be considered as well.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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