



Neuroblastoma in relation to joint effects of vitamin A and maternal and offspring variants in vitamin A-related genes: A report of the Children's Oncology Group



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ABSTRACT

Background: There is evidence vitamin A plays a role in neuroblastoma. Not only is 13-*cis*-retinoic acid used as maintenance therapy for high-risk cases, but prenatal vitamin intake use may decrease neuroblastoma risk. We hypothesized that single nucleotide polymorphisms (SNPs) in vitamin A-related genes are may be associated with neuroblastoma risk and potentially be modified by vitamin A intake.

Methods: The Neuroblastoma Epidemiology in North America (NENA) study recruited 563 case-parent sets through the Children's Oncology Group's Childhood Cancer Research Network. We ascertained dietary nutrient intake through questionnaires and genotyped 463 SNPs in vitamin A-related genes from saliva DNA. Offspring and maternal log-additive risk ratios (RR) and stratum-specific RR for gene-environment interaction were estimated with a log-linear model. We avoided false positives due to multiple testing by using the false discovery rate (FDR).

Results: When all neuroblastoma cases were considered together, no offspring variants met the significance criteria (FDR Q-value < 0.2). One maternal SNP (rs12442054) was associated with decreased risk of neuroblastoma (RR: 0.61; 95% Confidence Interval (CI): 0.47–0.79, Q = 0.076). When the cases were categorized according to prognostic risk category and age at onset, nine offspring SNPs were significantly associated with intermediate-risk neuroblastoma. Maternal rs6776706 was associated with (RR: 0.49; 95% CI: 0.33–0.72, Q = 0.161) high-risk neuroblastoma and maternal rs11103603 (RR: 0.60; 95% CI: 0.45–0.79, Q = 0.127) was associated with neuroblastoma aged < 1 year. For gene-environment interaction, maternal rs729147 was associated with decreased risk of neuroblastoma among mothers with vitamin A consumption above the recommendation.

Conclusions: Although there is biologic plausibility for the role of vitamin A in neuroblastoma, we found weak evidence of a relationship between vitamin A related genes and neuroblastoma.

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1. Introduction

Neuroblastoma is an extracranial cancer of the neural crest, which is a neural structure found in embryos and fetuses [1,2]. Since neuroblastoma has an embryonic origin, its etiology is likely random replicative errors with a combination of the prenatal environment interacting with offspring and maternal genetics. Genome-wide association (GWA) studies and studies of familial cases have identified common and rare germline variants associated with the risk of neuroblastoma [3–5].

Previous epidemiologic studies have found evidence of an inverse association between maternal prenatal vitamin use and neuroblastoma [6,7]. This suggests inadequate maternal pregnancy vitamin intake may play a role in neuroblastoma development. Beta-carotene, a common vitamin A component in prenatal vitamins, is essential for proper nervous system development [8]. Importantly, when cultured neuroblastoma cells are treated with retinoic acid – a metabolite of vitamin A – they exhibit decreased proliferation and increased differentiation [9]. However, when 13-*cis*-retinoic acid was used therapeutically to prevent recurrence in severe cases of neuroblastoma, the evidence of benefit was weak [8].

Considering the importance of vitamin A in neuronal development and differentiation as well as the epidemiologic associations between vitamin use and neuroblastoma, we hypothesize that common maternal and offspring SNPs in genes involved in vitamin A metabolism and transport are associated with neuroblastoma. Furthermore, we hypothesized that the effects of these variants are modified by maternal vitamin A intake through diet and prenatal vitamin supplementation.

2. Methods

2.1. Study subjects

The Neuroblastoma Epidemiology in North America (NENA) study used a case-parent triad design [10]. Details about the study have been previously published [11]. Briefly, we identified potential case families from the Childhood Cancer Research Network (CCRN), a registry system of newly-diagnosed cases who had agreed to be contacted for future research carried out by the Children's Oncology Group (COG) [12]. NENA approached 930 eligible families who had had a child diagnosed with neuroblastoma before the age of 6 years from December 24, 2007, to July 31, 2013, at a U.S. or Canadian COG institution. The biological mother had to be alive and willing to participate. The University of North Carolina at Chapel Hill (UNC) Institutional Review Board approved this study.

Our group sent study materials were sent to 870 families who agreed to enroll. We sent saliva samples from 618 (71.0%) mothers, 585 (71.4%) living children, and 520 (70.1%) fathers for genotyping. We were able to acquire a previously collected blood DNA sample from the COG Neuroblastoma Bio-repository at the Children's Hospital of Philadelphia (CHOP) for 19 deceased cases. Four of the 630 returned questionnaires were either incomplete or did not have a signed consent form, leaving 626 questionnaires for analysis. Detailed information about sample collection was previously reported [11].

2.2. SNP selection and genotyping

We selected genes important in the transport or metabolism of vitamin A. For efficiency, we used TAGster to choose one tag SNPs to represent each haplotype, based on high linkage disequilibrium (LD) ($r^2 \geq 0.8$), for genotyping. Since most of the mothers self-identified as white, we used the HapMap 3 release III, CEU population to tag SNPs. We only included SNPs with a minor allele frequency greater than or equal to 5%. To be inclusive and to capture distal promoter and enhancer sites, we tagged regions between 20 kb upstream to 10 kb downstream from a candidate gene. Since the case-parent triad design relies on transmission distortion and is not subject to confounding by

population stratification, ancestry-informative markers were not needed [13]. We selected 30 vitamin A related-genes, resulting in 484 haplotype tagging SNPs for genotyping.

The UNC Biospecimens Processing Facility completed DNA extraction and amplification. Samples with DNA yields less than 2 µg were excluded. UNC's Mammalian Genotyping Core Facility genotyped a total of 465 triads, 94 mother-child dyads, 4 father-child dyads and 61 others (mother-father dyads and singleton cases) using the Illumina GoldenGate Array.

For quality control, a Centre de l'Étude du Polymorphisme Humain (CEPH) family triad and blinded duplicates were included on each plate. We excluded SNPs with a genotyping call rate less than 95%. Individual genotypes for SNPs showing overlapping clusters in the raw genetic intensity data or showing apparent Mendelian errors in a particular family were treated as missing. A total of 426 vitamin A-related SNPs passed quality control. We assessed Hardy-Weinberg (HWE) equilibrium among parents who self-identified as white using chi-square tests in PLINK (v1.07) and flagged but did not exclude, SNPs that failed HWE at a false discovery rate (FDR) significance level of less than 0.2 ($n = 5$).

Individuals with genotyping rates < 95% or gender discrepancies were excluded. We confirmed relatedness for each triad through measures of identity by descent. When non-paternity was detected, the paternal data were excluded. A total of 465 triads, 94 mother-child dyads, 4 father-child dyads and 61 others (mother-father dyads and singletons) passed genetic quality control.

2.3. Biological and clinical variables

We obtained clinical and biologic characteristics of the tumor such as tumor genetics and stage from the COG Statistical and Data Center for all cases enrolled in a COG clinical protocol. Cases not enrolled in a clinical COG protocol ($n = 89$) were excluded from the risk-classification stratified analysis. We are interested in the COG prognostic "risk-classification" variable, which uses a schema based on pathology, tumor stage, *MYCN* amplification, ploidy, and age at diagnosis dichotomized at one year. Neuroblastoma cases are classified into low-risk, intermediate-risk, and high-risk prognostic groups [14].

2.4. Maternal vitamin use and diet

Mothers were asked about multivitamin or prenatal vitamin supplementation (excluding single vitamin use) 1-month pre-pregnancy and within each trimester of the case pregnancy. To minimize misclassification, we also asked about single vitamin use as a separate question. However, no women reported taking a single vitamin with only vitamin A. Since we are most interested in vitamin intake pre-pregnancy and during early pregnancy, we focused on prenatal vitamin or multivitamin use 1-month before pregnancy.

We ascertained the current and usual maternal diet during the preceding year using a self-administered semi-quantified food frequency questionnaire (FFQ) called the Dietary History Questionnaire (DHQ). We assumed maternal diet in the last year approximates pre-pregnancy diet. Completed FFQs were processed in Diet*Calc (version 1.5.0) to derive usual nutrient intake per day for the previous year.

We excluded questionnaires that reported calories per day below the 5th percentile ($N = 31$; below 854.47 calories) or above the 97th percentile ($N = 18$; above 4,508.75 calories). Vitamin A was estimated in micrograms (µg) retinoic acid equivalents (RAE), which accounts for the differing bioactivities of retinol and provitamin A carotenoids. We explored two cutoffs – one data-based and another based on recommendations – for vitamin A: 25th percentile (460.94 µg RAE) and recommended dietary allowance (RDA) for women of child-bearing age (700 µg RAE) [15].

We analyzed diet plus supplements combining prenatal vitamin or multivitamin use with nutrients from the diet. Maternal total exposure

was dichotomized as low intake and sufficient intake. To increase power for the interaction analysis, we categorized women with intake below the 33rd percentile of vitamin A from diet (532.51 µg RAE) and no prenatal or multivitamin supplementation 1-month pre-pregnancy as low intake. A woman was classified as sufficient intake if she had higher than the 33rd percentile of vitamin A from diet and/or took a prenatal or multivitamin supplement 1-month pre-pregnancy.

2.5. Statistical analysis

We used the previously described log-linear model to assess the offspring and maternal log-additive genetic main effects, to carry out stratified analysis, and to assess gene-environment interaction [13,16,17]. Additional description of the methods can be found in the appendix.

The maternal genotype and offspring genotype log-additive RRs were calculated simultaneously and thus are mutually adjusted. We used the expectation-maximization algorithm to allow inclusion of families with missing paternal genotypes [18]. Additionally, the case-parent triad design enables estimation of effects of genotypes and assessment of interactions between SNPs and the measures of vitamin A exposure.

For stratified analyses, offspring and maternal genetic models were fit for subsets of families based on their prognostic COG risk-classification and offspring age at diagnosis dichotomized at one year. Since age at onset is clinically relevant for neuroblastoma, we analyzed the “infant cases” separately, being those who were younger than one year at diagnosis, while “childhood cases” are the remainder. Cases with age of diagnosis less than one year tend to have cancers that present differently than those with older age of diagnosis [19].

The gene-environment interaction model allows the genotype-specific RRs to differ across levels of vitamin intake, which is equivalent to allowing an effect of vitamin intake that differs across genotypes [16]. The main genotype effects were coded co-dominantly, while the interaction term was fit additively to enhance power. If interaction terms were significant after correction for multiple testing, the interaction model was refit co-dominantly to characterize the interaction in a more flexible fashion.

All findings were corrected for the number of tests performed by calculating a false discovery rate (FDR) [20]. For a finding to be considered noteworthy, the FDR-corrected Q-value had to be less than 0.2, signifying a false positive rate of 20%. These results are presented within the main text. Results from the offspring and maternal models are reported in the appendix.

2.6. CHOP/COG replication study

We conducted a replication of our findings for offspring genotypes using an ongoing GWA study conducted at CHOP [21], which included 2101 European-American neuroblastoma cases and 4202 European-American healthy matched controls. This study has been described elsewhere [21]. To avoid overlap, cases who had also been enrolled in NENA were excluded from this analysis, resulting in 2052 cases and 4104 matched controls.

Imputation for unmeasured SNPs was performed on all case-control GWA data with IMPUTE2 using the worldwide 1000 Genomes Project Phase 1 Release 3 data as reference [22]. SNPs with an info score for imputation less than 0.8 were excluded. We used the available SNPs (N = 387) among those that we had used for the NENA case-parent analysis for testing the case-control association, adjusting for population stratification with principal component scores using SNPTTEST under an additive model [23]. About a third (n = 128) of the NENA SNPs required imputation for the COG/CHOP replication study. Odds ratios (ORs) were compared with the RRs from the NENA study.

2.7. Sensitivity analysis

During pregnancy, women tend to increase consumption of milk, thereby increasing their consumption of vitamin A. To address potential differences between usual diet in the past year (the question in the questionnaire) and their former diet during pregnancy, the questionnaire asked if the mother’s dairy intake during pregnancy was “Much less than it is now”, “Somewhat less than it is now”, “Same as it is now”, “Somewhat more than it is now”, and “Much more than it is now”.

We performed sensitivity analyses adjusting vitamin A nutrient levels depending on the self-reported change in dairy intake due to pregnancy. After this correction, vitamin A was dichotomized at the 25th percentile, and the gene-environment model was fit again. Also, since women who breastfeed are advised to consume more calories, additional sensitivity analyses were done excluding women who were breastfeeding at the time of the questionnaire administration (N = 47).

3. Results

We had genetic data for 465 triads and 98 dyads, which is the analytic set for the genetic model. Fig. 1 details the sample size available for each set of analyses. Table 1 displays the descriptive statistics for triads and dyads with genetic data, stratified by the RDA (700 µg RAE). Mean offspring age at diagnosis was slightly older (1.79 years) among babies of mothers with vitamin A intake below the RDA than among those with intake above the RDA (1.57 years). As expected, most mothers were white (84.7%).

3.1. Offspring genetic results

No offspring SNPs were significantly associated with neuroblastoma (Appendix Table A1). Nine SNPs were significantly (FDR < 0.2) associated with intermediate-risk neuroblastoma (Table 2). These nine SNPs are located near or in *RXRA*, *ADH1A*, *RARG*, and *ALDH1A2*.

3.2. Maternal genetic results

Maternal rs12442054 – proximal to *STRA6* – was significantly inversely associated with neuroblastoma (RR for each A allele: 0.61; 95% Confidence Interval (CI): 0.47–0.79; Table 3). In the stratified analysis, maternal rs6776706 was significantly associated with decreased risk of high-risk neuroblastoma (RR for each A allele: 0.49; 95% CI:

	Trio	Mother-Child Dyads	Father-Child Dyad	Other
Full genetic analysis	465	94	4	61
Vitamin A interaction analysis	458	89	3	55
Maternal effect				

Fig. 1. Sample size for each analysis.

Table 1
Descriptive statistics for triads and dyads included in the analysis with genetic data.

	Total		Below 700 µg RAE		Above 700 µg RAE		p-value ^a
	N	Mean (Std)	N	Mean (Std)	N	Mean (Std)	
Age at diagnosis (Yrs)	563	1.7 (1.42)	257	1.79 (1.42)	294	1.57 (1.40)	0.069
Maternal Age (Yrs)	551	29.3 (5.27)	257	29.71 (5.31)	294	28.86 (5.21)	0.059
	N	%	N	%	N	%	
Offspring gender							
Female	262	46.5	121	47.1	136	46.3	0.914
Male	301	53.5	136	52.9	158	53.7	
Maternal race							
White	466	84.7	219	85.5	247	84	0.371
Black	19	3.5	11	4.3	8	2.7	
Hispanic	35	6.4	12	4.7	23	7.8	
Other	30	5.5	14	5.5	16	5.4	
Missing	13		1		0		
COG risk-classification							
Low-risk	160	35.2	70	33.2	90	36.9	0.491
Intermediate Risk	129	28.4	58	27.5	71	29.1	
High-risk	166	36.5	83	39.3	83	34	
Missing	108		46		50		
Prenatal or multivitamin 1 month pre-pregnancy							
No	217	40.5	83	33.3	134	46.7	0.002
Yes	319	59.5	166	66.7	153	53.3	
Missing	27		8		7		

Yrs: Years; Std: Standard Deviation.

^a Comparing low vitamin A and high vitamin A.

0.33–0.72). For infant neuroblastoma, the log-additive risk ratio for rs11103603 was 0.60 (95% CI: 0.45–0.79) for each maternal C allele. Maternal results for all the SNPs are in Appendix Table A2.

3.3. Gene-environment interaction

We found a significant interaction between maternal rs729147 and maternal vitamin A intake dichotomized at the RDA (700 µg RAE) (Interaction p-value < 0.001; Q-value = 0.156). We then modeled the interaction (Fig. 2) co-dominantly to allow more flexibility when estimating RRs. When maternal vitamin A intake was below the RDA, one G allele of maternal rs729147 was significantly associated with increased risk of neuroblastoma (RR G/A vs. A/A: 1.49; 95% CI: 1.04–2.13) and two G alleles were suggestively associated with higher risk of neuroblastoma (RR G/G vs. A/A: 1.64; 95% CI: 0.40–3.58). When maternal intake was above the RDA, one or two G alleles were associated with a decreased risk of neuroblastoma (RR for G/A vs. A/A: 0.58; 95% CI: 0.38–0.87 and RR G/G vs. A/A: 0.51; 95% CI: 0.26–1.03). The maternal rs729147 was also significant for diet plus supplements with very similar point estimates, but wider confidence intervals due to low numbers carrying variant alleles in the low intake group (Appendix Fig. A1).

3.4. Replication study

Since maternal genotype and questionnaire data were not available from CHOP/COG, we were only able to compare offspring genetic results, not maternal genotypes or gene-nutrient interaction. Similar to NENA’s findings, none of the offspring SNPs from CHOP/COG were significant (Supplemental Table S1), including results within strata defined by risk-classification and offspring age at diagnosis. The

Table 2
Offspring additive risk ratios for FDR-corrected significant SNPs for intermediate risk group.

Gene	SNP	NENA				CHOP							
		Minor Allele	Major Allele	MAF	RR ^a (95% CI)	P-value	FDR Q-value	Minor Allele	Major Allele	MAF	OR ^b (95% CI)	P-value	FDR Q-value
RXRA	rs4842196	C	A	0.27	1.97 (1.32, 2.93)	0.001	0.185	C	T	0.22	0.87 (0.67, 1.12)	0.278	0.933
ADH1A	rs1229977	T	C	0.21	0.48 (0.31, 0.75)	0.001	0.185	T	C				
RXRA	rs1045570	T	G	0.17	2.07 (1.32, 3.24)	0.002	0.185	T	C				
RXRA	rs1007971	G	C	0.23	1.94 (1.27, 2.97)	0.002	0.185	T	C				
RARG	rs7139068	T	A	0.10	0.40 (0.21, 0.73)	0.003	0.185	T	C				
ADH1A	rs904092	A	G	0.18	0.48 (0.29, 0.78)	0.003	0.185	A	A	0.17	0.74 (0.56, 0.98)	0.038	0.933
RXRA	rs3118523	G	A	0.21	2.09 (1.27, 3.43)	0.004	0.185	T	C				
ALDH1A2	rs7169439	A	G	0.11	2.75 (1.39, 5.45)	0.004	0.185	T	C	0.09	1.10 (0.75, 1.62)	0.609	0.933
RARG	rs1465057	C	T	0.11	0.37 (0.19, 0.73)	0.004	0.185	C	T				

MAF: Minor Allele Frequency; CHOP: Children’s Hospital of Philadelphia case control validation study; RR: Risk Ratio; CI: Confidence Interval; OR: Odds Ratio; -: Unavailable in validation study.
^a Minor allele is the allele with respect to which the RR is calculated.

Table 3
Maternal additive risk ratios for FDR-corrected significant SNPs.

SNP	Gene	RR ^a (95% CI)	P-value	Q-value
Overall				
rs12442054	STRA6	0.61 (0.47, 0.79)	< 0.001	0.076
High-Risk				
rs6776706	RARB	0.49 (0.33, 0.72)	< 0.001	0.161
Infants				
rs11103603	RXRA	0.6 (0.45, 0.79)	< 0.001	0.127

RR: Risk Ratio; CI: Confidence Interval.

^a Minor allele is the allele with respect to which the RR is calculated.

significant NENA results for intermediate-risk neuroblastoma that were available in CHOP/COG did not replicate (Table 2) in the CHOP case-control study.

3.5. Sensitivity analysis

Among women with intake below the RDA for vitamin A (700 µg RAE), 29 women reported significantly increasing dairy intake during pregnancy compared to current diet. When these women were re-classified into a higher quartile of vitamin A consumption, the maternal rs729147 interaction remained significant. After breastfeeding mothers were excluded (N = 47), no SNPs were significant, but the point estimate for rs729147 trended in the same direction.

4. Discussion

We analyzed the association between maternal and offspring SNPs in vitamin A-related genes and neuroblastoma as well as gene-environment interactions with maternal vitamin A intake. Overall, we found no offspring SNPs associated with the risk of neuroblastoma, but we found associations between neuroblastoma and several maternal SNPs. Additionally, one maternal SNP was found to interact with vitamin A consumption based on the RDA to affect neuroblastoma risk.

We found the T allele of maternal rs6776706 located in an intron of RARB was associated with decreased risk of high-risk neuroblastoma. There was little evidence for this SNP in relation to either low-risk (RR: 1.17; 95% CI: 0.84, 1.64) or intermediate-risk (RR: 1.23; 95% CI: 0.82, 1.83) neuroblastoma. However, the function of this SNP and how this SNP relates to high-risk neuroblastoma is unknown. Although these are established clinically prognostic risk categories, genome-wide association studies have found distinct variants associated with high-risk neuroblastoma, suggesting this prognostic category may also have etiologic relevance [3,24]. Additionally, there is little evidence that favorable tumors progress to unfavorable tumors [25].

The T allele of maternal rs11103603, almost 10 kbp downstream from RXRA, was associated with a decreased risk of neuroblastoma in infants (RR: 0.60; 95% CI: 0.45–0.79). This maternal variant was not

associated with neuroblastoma in children older than one year (RR: 1.07; 95% CI: 0.84–1.37). The SNP rs9409929, in high linkage disequilibrium with rs11103603 ($r^2 = 0.898$), has been previously reported to be associated with increased levels of calcitriol – a hormonally active vitamin D metabolite [26]. Thus, maternal rs11103603 is associated with decreased risk of neuroblastoma in infants, but also with increased levels of maternal vitamin D. This link is biologically plausible since vitamin D levels have been previously associated with reduced cancer risk [27]. However, additional studies are needed to elucidate the relationship between vitamin D levels and neuroblastoma in infants. Unfortunately, since NENA did not collect blood samples, we are unable to address this directly.

We found one significant result for a maternal gene-vitamin A interaction when vitamin A was dichotomized at the RDA value. This variant (rs729147) is 500bp downstream from ADH7, which encodes a gene that converts retinol to retinoic acid. ADH7 is involved with alcohol metabolism, and rs729147 has been previously studied, but no association was found with alcoholism [28,29]. However, since we were quite inclusive with our genotyping and this SNP has never been examined with vitamin A processing and transport and will merit additional studies.

This present study has a few limitations. Since the neural crest starts responding to differentiation signals at five weeks, our exposure window of interest is early pregnancy and pre-pregnancy [30]. We are using post-pregnancy usual diet as a proxy for this period, and those data are subject to measurement error. We also explicitly queried mothers on changes in diet due to pregnancy to mitigate measurement error. A few studies have reported that maternal nutrition tends not to shift drastically during pregnancy [27,31], but it is possible that current diet does not entirely reflect diet in early pregnancy, but rather a pre-pregnancy diet before morning sickness. We also conducted a sensitivity analysis altering vitamin A due to dairy changes, which suggested the association was not markedly affected by diet changes due to pregnancy. The mothers were interviewed during a time when their child might have been suffering from a critical illness, which could have substantially disrupted their routine behavioral patterns or influenced their reporting. However, we found little change in vitamin A consumption by risk-classification, a measure of severity of neuroblastoma. There is also the strong potential for misclassification of prenatal and multivitamin use. However, in a previous study, the sensitivity of recall of vitamin use was 84% after 30 years (while in our study the average time from questionnaire completion to conception was 3.1 years) [32]. Additionally, since there are no controls, the recall misclassification should be unrelated to the alleles.

Of the 930 eligible cases, we received genotyping data from 66.5% mothers and 62.9% offspring and survey data from 67.3%. Although our response rate is higher than that for many contemporary studies, non-participation could affect the external and internal validity of our study [33]. However, it is unlikely that participation depended on genomic data and thus unlikely a source of bias.

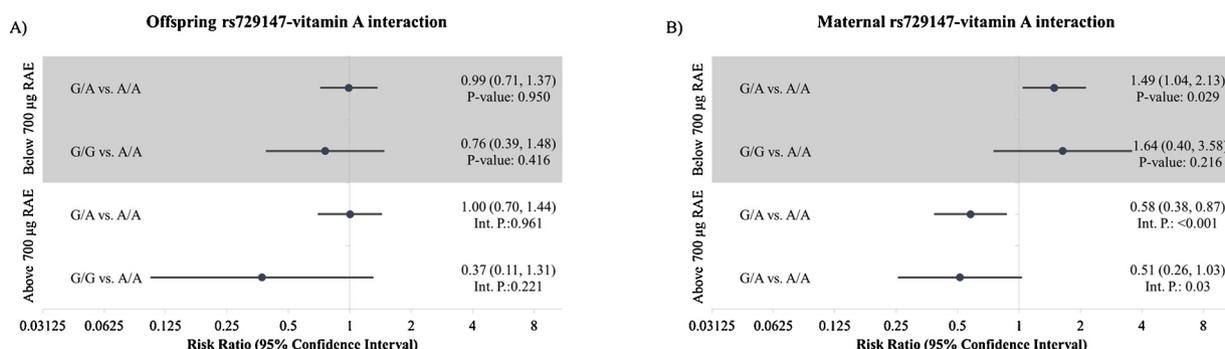


Fig. 2. A) Offspring and B) Maternal rs729147-vitamin A interaction fit co-dominantly with maternal vitamin A intake dichotomized at the RDA (700 µg Retinoic Acid Equivalents) where the minor allele is the allele with respect to which the RR is calculated.

We believe we had excellent coverage for genes using SNPs selected from the CEU population, but a small proportion of the participants (93 mothers) are non-white. Consequently, the gene coverage for the non-white families was not ideal. Violation of the assumption of mating symmetry in relation to the loci under study (i.e., the alleles of the mothers can be validly compared with the fathers) can occur if there is uneven pairing by race, and those racial asymmetries can produce spurious maternal associations. However, when the non-white families and mixed-race families were excluded, the point estimates of the main genetic model were minimally affected, suggesting that there is no substantial violation of this assumption. Unfortunately, we did not have adequate sample size to restrict for the interaction analyses.

This study also had notable strengths. Vitamin A has strong biologic plausibility in the context of the etiology of neuroblastoma and is essential for the differentiation of neuronal cells. Since less differentiated neuroblastoma tumors present in a more aggressive state, 13-*cis*-retinoic acid is commonly used as maintenance treatment in conjunction with antibody therapy [8]. Vitamin A is transferred from the mother to the placenta, highlighting the role of maternal genetics in fetal development and subsequent neuroblastoma malignant transformation [34]. We also have a case-control partial replication study from CHOP to try to confirm our results further. Our use of the case-parent triad approach allowed for the assessment of maternal genetic effects.

Additionally, this is the most extensive study to date with both genetic and maternal questionnaire data to enable the study of gene-environment interaction. The case-parent triad approach eliminates the need for a control group [13,16]. The Children's Oncology Group is the primary resource to collect a large number of cases. However, to collect population-based controls for a North-America-wide study would have presented a logistical and validity challenge. The case-parent triad design is robust against bias due to population stratification and bias due to self-selection based on ethnicity.

5. Conclusion

In conclusion, we targeted variants in genes from the vitamin A pathway and did not find strong evidence that genetic variants related to vitamin A metabolism and transport play a role in neuroblastoma etiology. Although we saw a few associations with maternal SNPs, due to the uncertain functionality of these SNPs and the fact that some of the associations were found only in subphenotypes of neuroblastoma, replications of these results will be needed.

Author's contribution

Conception and design of study: AFO, JMM; acquisition of data: KSC, PVB, ZV, AMSR, SJD; analysis and/or interpretation of data: ALM, CRW, SME, AMSR, FZ.

Drafting the manuscript: ALM; revising the manuscript critically for important intellectual content: ALM, CRW, SME, AMSR, FZ, KSC, PVB, ZV, JMM, SJD, CM, AN, AFO.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.canep.2019.06.009>.

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