Prenatal Autoimmune Risk Factors in the Mother with Brain-reactive Antibodies for Neonatal Outcome.

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Abstract:

Background: The increased number of childbearing women with autoimmune diseases leads to a growing interest in studying the relationship between maternal autoreactivity, pregnancy, and the development of offspring. The aims of this study were to provide a descriptive analysis of the Generation ECHO neonatal cohort and determine the impact of maternal autoreactivity on neonate outcome.

Methods: The study population was drawn from the Generations Project-Environmental influences on Child Health Outcomes (ECHO) project. The current study focused on analyzing data pertaining to neonate outcome by sex and neonate outcome with respect to ANA and anti-brain Ab prevalence which was recorded during the Generation ECHO project and stored in REDCap. Data analyses such as the Student *t* test or Mann–Whitney and analysis of variance (ANOVA) or Kruskal-Wallis test, if more than two groups were involved, were adequately conducted. All tests were two- tailed. P values ≤ 0.05 were considered statistically significant. Data analyses were performed using Microsoft Excel and SPSS statistical package (version 12; SPSS Inc., 2009, Delaware, USA).

Results: There was no significant difference for gestational age between M vs F neonates (p=.41). There was a significant difference between birth weight (p=.00) and head circumference (p=.04) for M vs F neonates and there was a trend towards significance for birth length (p=.08) for M vs F neonates. 37.8% (N=165) of mothers tested positive for ANA while 27.5% (N=120) of mothers tested positive for anti-brain Ab. There was a significant difference in gestational age of neonates (p=.030) and birth weight of neonates (p=.033) between mothers with and without the presence of ANA Ab. There was no significant difference in neonatal head circumference or neonatal length at birth if these neonates had mothers with or without the presence of ANA Ab. There was no significant difference between an offspring's gestational age, birth weight and birth length and whether their mothers harbored anti-brain Ab. There was a trend toward significance in head circumference of neonates with mothers who tested positive for anti-brain Ab (p=.070).

Conclusions: Maternal autoreactivity, especially maternal exposure to ANA should be carefully monitored during pregnancy to ensure the best possible management of mothers, fetuses and newborns.

Abbreviations: ANA= antinuclear antibodies; Ab= antibody or antibodies REDCap= Informational Technology Source

I. Introduction

The possibility that autoimmune mechanisms are a contributing factor in Autism Spectrum Disorder (ASD) has been entertained for decades, ever since early studies proposed that autoimmune diseases are common in the family histories of individuals with ASD (Itzchak, 2012 and Keil, 2010). ASD is reported to affect 1 in 67 live births in the United States. The dramatic increase in the prevalence of ASD is supported by several studies, including the CDC which reported a 78% increase in ASD between 2002 and 2008 (Itzchak, 2012) and data from the National Health Interview Survey which recorded that from about 1 in 6 children in the United States had a developmental disability in 2006–2008 (Boyle, 1997/2008). ASD is known to be more prevalent in males than females and the sex ratio falls to 3.25 boys per girl (Baron-Cohen-2011).

Autoimmune mechanisms refer majorly to autoantibodies, immune proteins that mistakenly attack the body's own cells. Antinuclear antibodies (ANA) are antibodies (Ab) which attack healthy proteins within a cell's nucleus; ANA are characteristic of many autoimmune diseases. Studies have found that ANA are elevated in the blood of mothers of children with autism who also carry anti-brain Ab, which are Ab in maternal blood that react with proteins in the brains of offspring (Brimberg, 2013), compared with those who do not have anti-brain Ab. This is consistent with the theory that autoimmunity predisposes mothers to having brain-reactive antibodies and giving birth to children with ASD (Diamond, 2016). Studies have shown that mothers of an ASD child are at least four times more likely to harbor anti-brain Ab (Brimberg, 2013).

Moreover, anti-brain Ab affecting the developing brain have been suggested to be the cause in a subset of Autism Spectrum Disorders. Maternal autoantibodies cross the placenta and enter the fetal brain, leading to alterations (Spectrum: Autism Research News, 2016). If Ab exposure occurs at a critical time of development, this can result in irreversible damage of the offspring that persists throughout adulthood (Mader, 2017). Additionally, a deficit to restrict self-reactive antibody production, or being deficient in immune tolerance, may be the foundation of self- reactive antibody production (Braunschweig, 2012).

Previous studies represent autoreactivity presence in mothers who have rheumatoid arthritis and celiac disease. This gives the possibility of a relationship between maternal autoreactivity, autoantibody prevalence, and ASD (Brimberg, 2013). In addition, in some cases, ASD is the consequence of immune activation in the mother leading to impaired fetal brain development (Mazzucchelli, 2015).

On the basis of previous studies, there are certain neonatal traits which are characteristic of neonates who have ASD. These involve a decreased birth weight (Ben Itzchak, 2011) a lower gestational age, or a more premature birth (Hatch, 2011), and a smaller head circumference (Elder, 2008) as well as a presumably smaller birth length.

Due to the stark increase in the number of childbearing women with autoimmune diseases, there is a growing interest to study the relationship between maternal disease, pregnancy, and the development of offspring (Itzchak, 2012). Since mothers who have autoimmune diseases harbor autoimmune mechanisms such as ANA and antibrain Ab, there is a strong reason to study the effects of maternal autoreactivity on neonate outcome.

The primary objective of the current investigation is to evaluate the impact of the presence of maternal autoreactivity on neonatal outcome in Generation's ECHO cohort data which was stored in REDCap. The data analyzed here is a subset of the Generation ECHO data set. Generation ECHO (Environmental influences on Child Health Outcome) is a longitudinal study which collects maternal and respective neonatal data to determine if autoreactive mothers have offspring which are at high-risk for ASD. This would be true if maternal autoreactivity leads to the production of anti-brain antibodies that can cross the placenta and the fetal blood brain barrier and alter neonatal outcome. Given this, the hypothesis for the current study, is that maternal autoreactivity, classified by the maternal harboring of anti-brain antibodies and antinuclear antibodies, alter neonatal outcome. In the current study, as per the neonatal outcome, four attributes were analyzed- birth weight, gestational age, head circumference and birth length- in regards to their linkage to maternal autoreactivity; for each of the three respective neonatal outcomes, a decreased value was hypothesized. Specifically, I predict that autoreactive mothers, mothers who test positive for harboring antinuclear and anti-brain Ab, have offspring with decreased values for their head circumference, birth weight, gestational age and birth length.

II. Methods

Study Population

The study population was drawn from the Generations Project-Environmental influences on Child Health Outcomes (ECHO) longitudinal sample collection and follow up at Northwell Health. The data utilized for this project was collected as part of Generations Project-ECHO of the Northwell Health. The Generation Project- ECHO is a prospective, population-based longitudinal study of approximately infants born at Northwell Health, and their biological mothers over the two year recruitment period. The original Generations Project-ECHO was a part of the National Institute of Health's initiative called the Environmental influences on Child Health Outcomes (ECHO) Program set to improve our understanding of the effects of environmental exposures on child health and development.

De-identified patient data was obtained from REDCap database. REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative

self-documenting process by all members of the research team with planning assistance from the Research Information Systems department and the Biostatistics Unit of the Feinstein Institute for Medical Research. The REDCap system provides secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data, and have real-time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and reporting for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). They also allow for HIPAA compliant collaboration across institutions and investigators. Only de-identified data was provided to the student for this project.

The current study focused on analyzing data recorded during the Generation ECHO project which was stored in REDCap, a secure web application for building and managing online surveys and databases. The study population for the current study encompassed both maternal and neonatal data. The data had been deidentified prior to being given to me. Names and ages of mothers were retracted from the original data set and GPE scores (randomly assigned six-digit numerical codes) replaced them. Data was then transferred to Microsoft Excel and then SPSS software to perform the necessary data analyses. There were 1,161 total participants in the data set. There were 586 mothers (ages 19-45) and 575 neonates. The current study performed descriptive statistics to describe the maternal and neonatal cohort. Firstly, this study displayed the demographic information of the neonates in two categories: race and ethnicity (Table 1).

Table 1: Cohort Description (Demographics-Neonates)

Race	Frequency (%)	Ethnicity	Frequency (%)
White	182 (31.7)	Hispanic	96 (16.7)
African American	120 (20.9)	Non-Hispanic	322 (56.1)
Native American	10 (1.7)	Not reported	156 (27.2)
Asian	140 (24.3)		
Other/Multiracial **Not Reported**	118 (20.6)\ 4 (.7)		

574 pregnant females elected to take part in the study. Data on race/ethnicity are described above.

The demographics represented a fairly diverse population which was reflective of the community at Northwell which is comprised of members primarily from Long Island, New York and Queens, New York.

To further describe the neonatal cohort, this study classified data on neonatal gestational age that was collected during Generation ECHO's project into two discrete categories- gestational age that was less than 37 weeks (w<37) and a gestational age that was greater than or equal to 37 weeks (w>/= 37) (Table 4). The former range of gestational ages can be deemed those that are characteristic of premature neonates (O'Driscoll, 2018). These neonates most likely required a stay in the NICU, while the latter group of neonates most likely did not require a stay in the NICU. This study additionally showed the amount (frequency) and proportion of neonates who either stayed or did not stay in the NICU (Table 5).

Table 4: Neonatal Prematurity (weeks)

Gestational Age (weeks)	Frequency (%)
<37 w	54 (9.4)
≥37 w gestation	512 (89.2
Not Reported	8 (1.4)

A small minority of neonates were born prematurely, after less than 37 weeks of gestation. A large majority of neonates were born maturely, at 37 or more weeks of gestation.

Table 5: NICU Stay

NICU Stay	Frequency (%)
No	417 (72.7)
Yes	113 (21.3)
Not Reported	44 (7.7)

A majority of neonates did not require a stay at the NICU while a minority did require a NICU stay.

The apgar score is one neonate outcome that was accounted in the study's methods. Original data for the apgar score encompassed values between one and ten since the apgar scores are based on a total score from 1-10 (MedlinePlus) and each score is scored on a scale from 0 to 2, with 2 being the best score (kidshealth). There are distinct attributes for each respective score under the five categories (Figure 1).

Figure 1: Apgar Scoring Technique (Park, 2015)

	0 (Points)	1	2
Appearance	Blue or pale all over	Blue extremities, but torso pink	Pink all over
Pulse	None	< 100	≥ 100
Grimace	No response	Weak grimace when stimulated	Cries or pulls away when stimulated
Activity	None	Some flexion of arms	Arms flexed, legs resist extension
Respirations	None	Weak, irregular or gasping	Strong cry

0-3 Critically Low, 4-6 Fairly Low, 7-10 Generally Normal

In order to further depict the health of the neonate in terms of their apgar scores, the current study classified the apgar scores which were recorded in the Generation ECHO project at 1 minute and 5 minutes postnatal, in three discrete categories: 0-3, 4-6, 7-10 (Table 3).

Table 3: 1 minute and 5 minute Apgar Scores

Apgar Scores (1 and 5 min)		
Apgar Score Range	**1 min Frequency (%)**	**5 min Frequency (%)**
0-3	15 (2.6)	2 (.3)
4-6	22 (3.8)	5 (.9)
7-10	525 (91.5)	554 (96.7)
Not Reported	12 (2.1)	13 (2.3)

There was no significant difference between M vs F in 1 min (p=.185) and 5 min (p=.165) Apgar scores by the student unpaired t-test. There was no significant difference in 1 and 5 min Apgar scores between mothers with and without the presence of ANA and with or without the presence of ABA by the student unpaired t-test.

Statistical Analyses

Neonate Outcome by Sex

The current study further classified neonatal data by sex: 284 (49.5%) females and 290 (50.5%) males. The variability (sd) and average (mean) were taken for the four neonatal outcomes and an unpaired t-test was performed to compare four distinct variables: gestationa age, birth mass, birth length and birth head circumference with respect to the sex of the neonate. The Student unpaired *t* test or Mann–Whitney test served to establish the level of significance between sex within each of the respective neonatal outcomes.

Significance was determined based on the calculated p-value with p values ≤ 0.05 being considered statistically significant (Table 2).

This study also performed Student *t* tests or Mann–Whitney tests to determine whether there was a significant difference between the 1 minute and 5 minute apgar scores between M and F and whether maternal autoreactivity- prevalence of ANA or anti-brain Ab- had an effect on neonatal apgar score.

Part of the maternal cohort's descriptive data was their values for ANA and anti-brain AB. The current study coded the values for the ANA and anti-brain AB prevalence into levels based on the AB value. ANA values<20were classified as negative. AB values between 20 and 60 were classified as moderate positive and Ab values >60 were classified as positive. For the anti-brain antibody classifications, there was an additional classification labeled 'indeterminate' which served to classify the number and proportion of mothers who's anti-brain antibody levels could not be determined. Although there were 586 mothers in total, information on the antinuclear AB levels of mothers totaled up to only 436 and information on the anti-brain AB levels totaled up to only 541 due to the difference in the total number of subjects tested for each antibody. In Generation ECHO's project, different types of methods were used to detect each antibody. Some samples may have worked for ANA and not worked for anti-brain Ab. In some situations, there was only enough of a sample to check one antibody and a decision was made to use it for the anti-brain antibody, rather than the antinuclear antibody. In other situations, samples needed to be re-run multiple times which prevented them from being used for both types of tests. The current study displayed the results of the autoreactivity levels in mothers after coding for each of the Ab levels (Table 6).

To fulfill the primary objective of the study, the descriptive data on maternal AB exposure was utilized to compare to neonatal outcome- specifically neonatal gestational age, birth weight, birth length and head circumference- with maternal autoreactivity (maternal anti-brain antibodies and/or anti-nuclear antibodies (ANA) exposed) to those mothers who do not have autoreactivity (unexposed). With respect to maternal anti-brain AB prevalence and ANA prevalence, the variability and averages of the four neonatal outcomes were assessed. In addition, an ANOVA test was performed to assess the relationship between maternal autoreactivity and neonatal outcome (Table 7).

III. Results and Discussions

A simple statistical analysis was performed with mean and variability (sd) values calculated for each respective neonatal outcome with respect to the sex of the neonate.

Table 2: Neonate Outcome by Sex

Neonate Outcome	Sex (M/F)	N	mean	sd
Gestational age (weeks)	M	286	38.9	1.8
Not reported: 8	F	280	38.8	1.9
Birth weight (g)	M	287	3311	592.8
Not reported: 4	F	283	3114.7	588.3
Length (cm)	M	269	50.2	4.3
Not reported: 49	F	256	49.6	3.8
Head Circ. (cm)	M	267	33.9	4.7
Not reported: 52	F	255	32.9	5.9

The N of neonatal F and males M, the mean values and sd values for gestation, birth weight, length and head circumference are depicted. There was no significant difference for gestational age between M vs F (p=.41). There was a significant difference between birth weight (p<.001) and head circumference (p=.04) for M vs F and there was a trend towards significance for birth length (p=.08) for M vs F.

This is consistent with previous findings that found that males are significantly heavier and have a greater head circumference than females ($p < 0.001$) at birth (Galjaard, 2019). Previous studies found that the male length at birth is significantly higher than female length at birth ($p < 0.001$) (Galjaard, 2019). This is partially consistent with the results of this study which found a trend towards significance for birth length (.08) between male and female neonates.

An unpaired t-test was performed to determine whether there was a significant difference between the 1 minute and 5 minute postpartum health of the neonate, which is depicted by their apgar score.

There was no significant difference between M vs F in 1 min and 5 min Apgar scores (p=.185 and p=.165 respectively).

Generally, a score of 7, 8, 9 or 10 is normal and is a sign that the newborn is in good health (MedlinePlus). Thus, results depicted that a majority of this neonate population, 93.4% at 1 minute postpartum and 98.7% at 5 minutes postpartum, was healthy. This is consistent with other findings which found that ___% (N) of () neonates had an apgar score from 7-10 and were healthy. Although there was no significant difference in apgar scores (p=.185 for one minute postpartum and p=.165 for five minutes postpartum) at 1 minute and 5 minutes postpartum, the increased number of neonates with higher apgar scores after five minutes is most likely due to the natural ability for a neonate to become healthier as they live more (). The insignificant differences between the 1 minute and 5 minute apgar scores are additionally consistent with previous findings which found that … ()

There was no significant difference in 1 and 5 min Apgar scores between mothers with and without the presence of ANA Ab. There was no significant difference in Apgar scores in 1 and 5 min Apgar scores between mothers with and without the presence of Anti-Brain Ab.

This study coded maternal antibody value ranges from Generation ECHO's data into qualitative levels.

Table 6: Frequency of Antinuclear and Anti-Brain AB

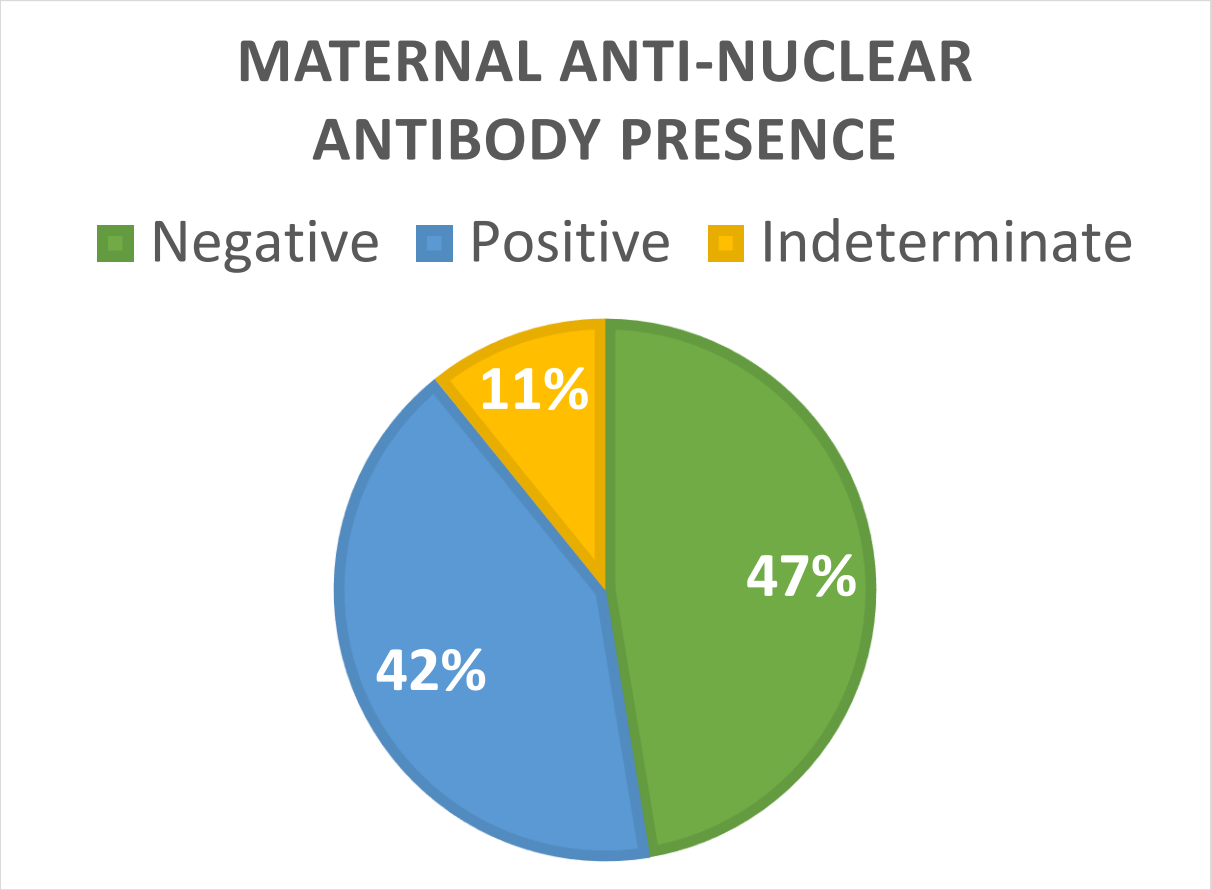
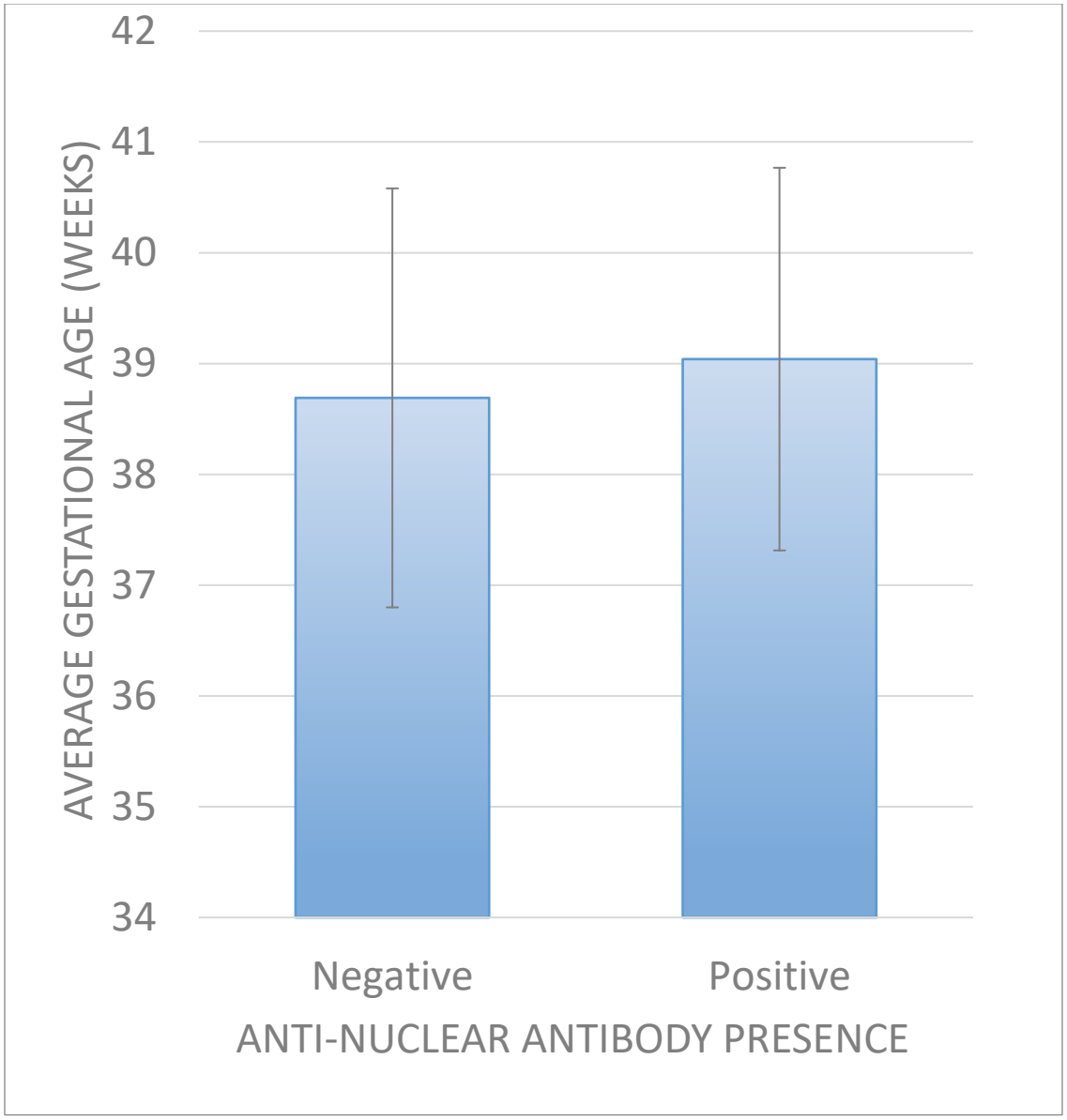
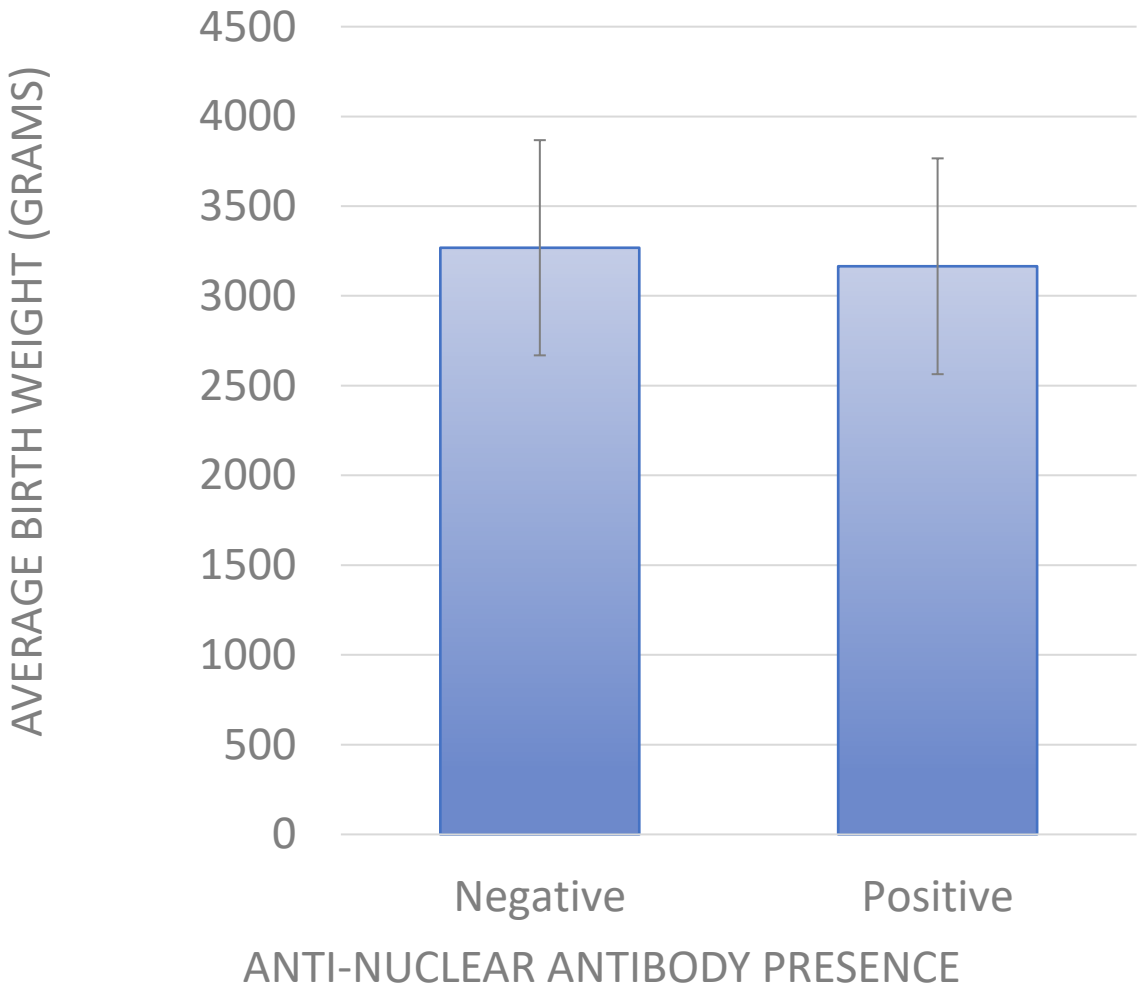


FIGURE 1

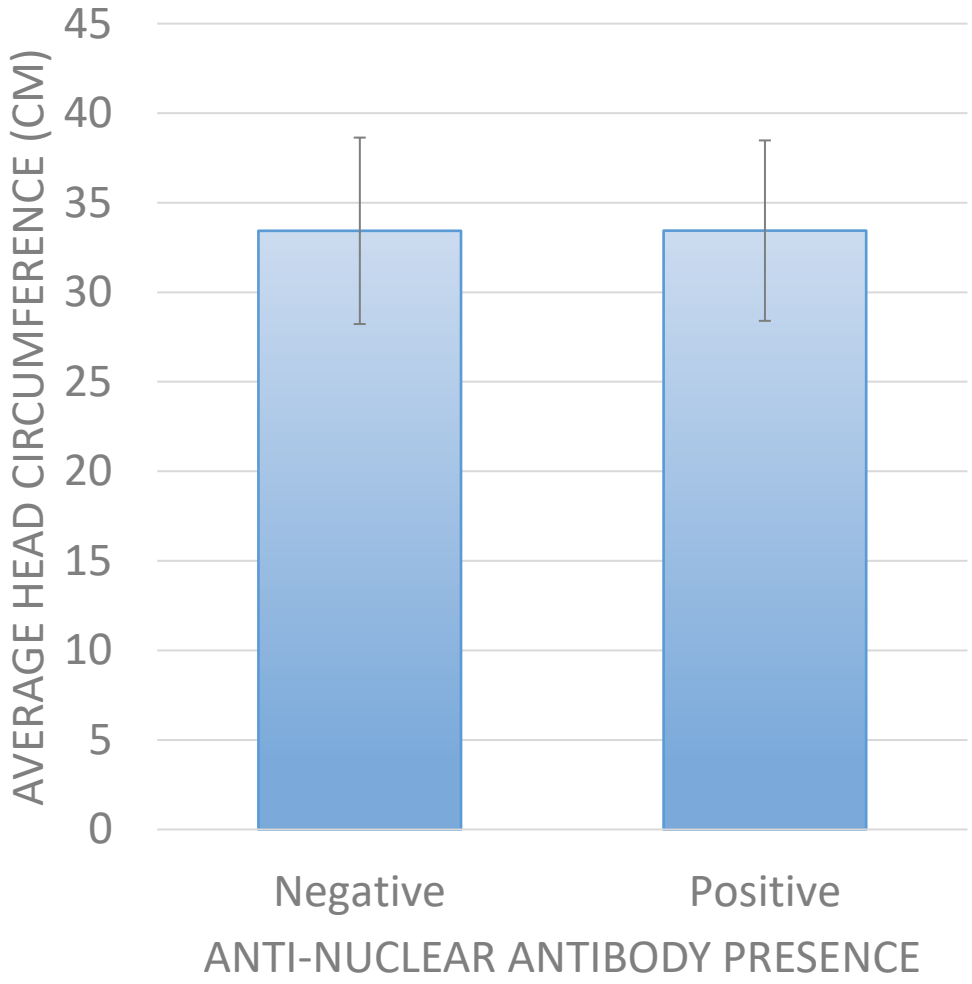
512/574 mothers were tested *and* received a measurable result for ANA presence. Values <20 were classified as negative. ANA values between 20 and 60 were classified as moderate positive and ANA values >60 were classified as strong positive. Data depicted that 42% (N=272) mothers tested positive for ANA.



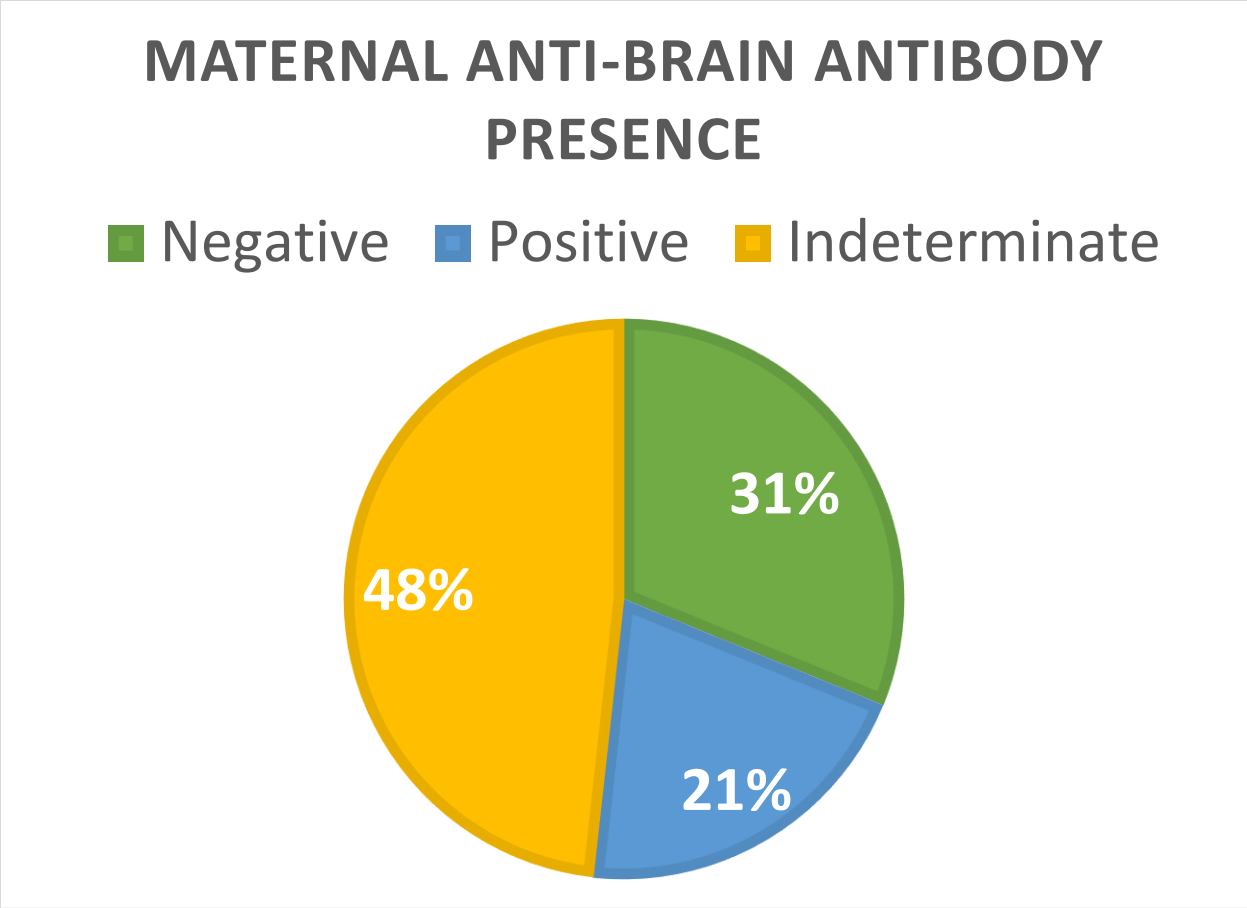
The effect of maternal ANA presence on average neonatal gestational age. Error bars represent +/- 1 standard deviation. p=.03 by the ANOVA assay.



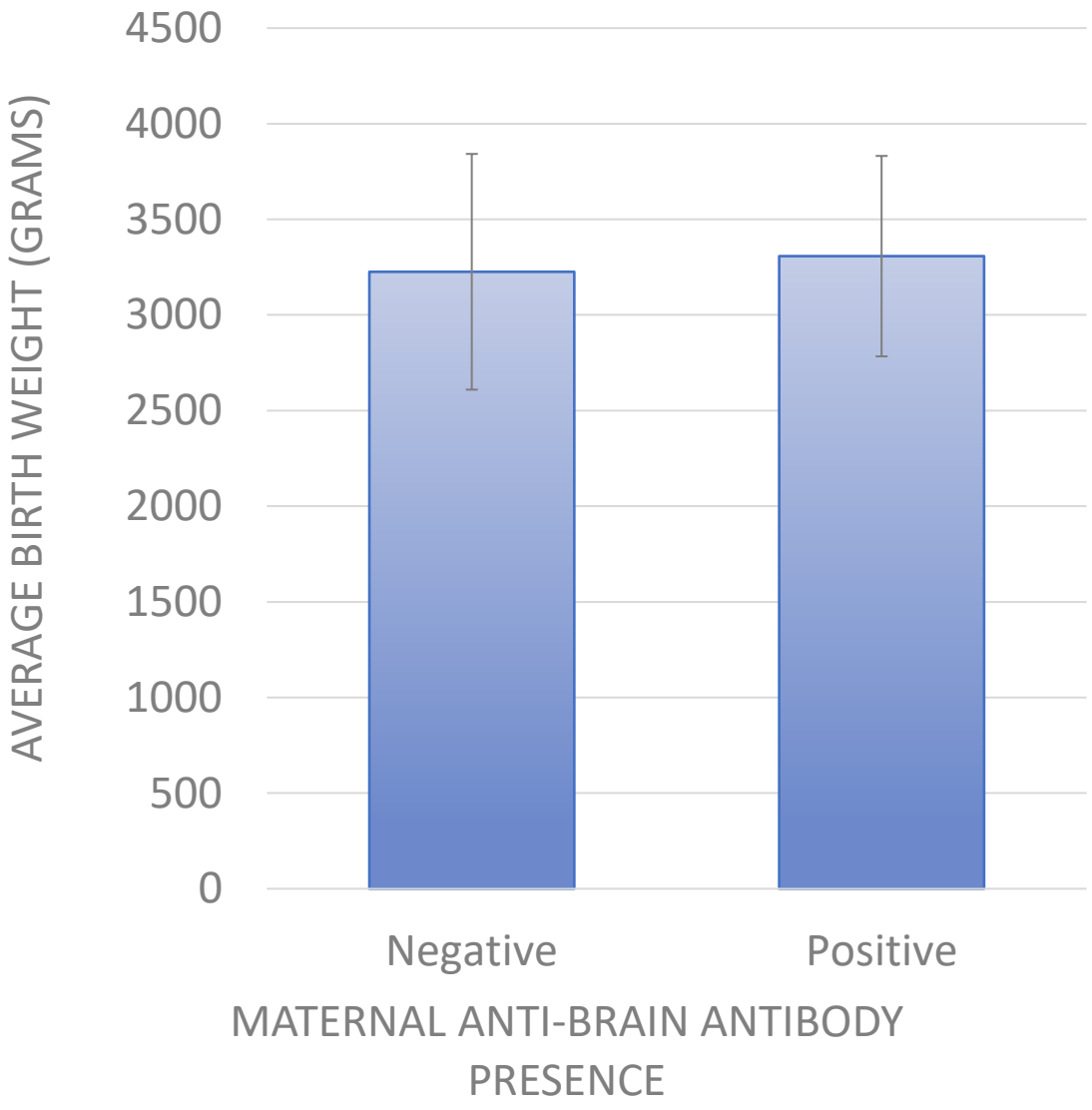
The effect of maternal ANA presence on average neonatal birth weight. Error bars represent +/- 1 standard deviation. p=.033 by the ANOVA assay.



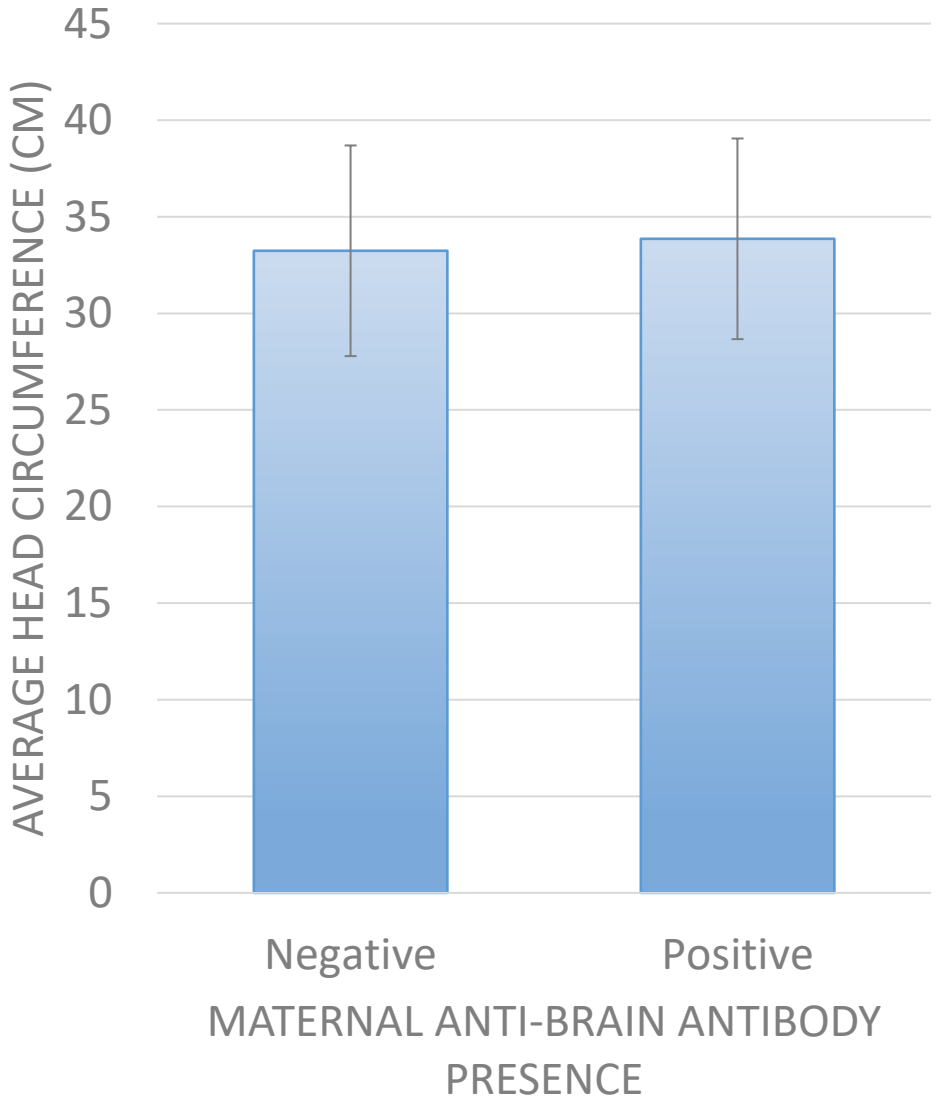
The effect of maternal ANA presence on average neonatal head circumference. Error bars represent +/- 1 standard deviation. $p \geq .05$ by the ANOVA assay.



297/574 mothers were tested *and* received measurable results for ABA presence. ABA values <20 were classified as negative. ABA values between 20 and 60 were classified as moderate positive and ABA values >60 were classified as strong positive. Data depicted that while 27.5% (N=120) of mothers tested positive for ABA.



The effect of maternal ABA presence on average neonatal birth weight. Error bars represent +/- 1 standard deviation. $p \geq .05$ by the ANOVA assay.



The effect of maternal ABA presence on average head circumference. Error bars represent +/- 1 standard deviation. P=.07 by the ANOVA assay.

Taking into account this data, an ANOVA test was then performed to determine whether there was significance between a mother harboring either the ANA or the anti-brain AB and their offspring's neonatal characteristics. Data depicts the five neonate outcome averages (mean) and measures of variability (sd) with respect to whether their mothers tested negative or positive for autoreactivity.

Table 7: Autoreactivity and Neonate Outcome

Anti-Brain Ab		N	mean	sd
Gestational age (wks)	Negative	174	38.69	1.989
	Positive	117	39.04	1.483
Birth weight (g)	Negative	177	3225.88	616.3
	Positive	117	3307.62	524.115
Length (cm)	Negative	171	49.951	4.0782
	Positive	109	50.435	2.863
Head circ (cm)	Negative	171	33.24	5.453
	Positive	109	33.86	5.193
ANA-Ab		**N**	**mean**	**sd**
Gestational age (wks)	Negative	267	38.98	1.891
	Positive	161	38.63	1.727
Birth weight	Negative	269	3268.28	599.413
	Positive	163	3165.14	601.175
Length (cm)	Negative	261	50.117	4.3205

Results revealed a significant difference in gestational age of neonates (p=.030) and birth weight of neonates (p=.033) between mothers with and without the presence of ANA Ab. There was no significant difference in neonatal head circumference or neonatal length between mothers with and without the presence of ANA Ab. There was no significant difference between an offspring's gestational age, birth weight and birth length and whether their mothers harbored anti-brain Ab. There was a trend toward significance in head circumference of neonates with mothers who tested positive for anti-brain Ab (p=.070).

IV. Conclusions

Data from Generation ECHO, the longitudinal study done to determine whether autoreactive mothers had offspring who were at high risk for ASD was collected, but there was no prior data analysis done. The current study served to assess the relationship between maternal autoreactivity and neonatal outcome which was defined, in this study, by four specific neonatal characteristics: gestational age, birth weight, birth length and head circumference. By performing statistical analyses on data that was collected in the Generation ECHO study, the current study served as the primary and preliminary data analysis done on the Generation ECHO data sets.

Although the sample size was large, a limitation was that it may be unrepresentative of a larger population- such as nationally or internationally- since it is data collected on mothers and their offspring

solely from Generation ECHO's cohort which was all mothers who had babies from April 1st, 2017 to August of 2019 from North Shore University Hospital and Long Island Jewish Medical Center at Northwell Health hospital. The data was drawn from sample collection and follow up at Northwell Health.

However, based on the performed analyses, significant results reveal that gestational age and birth weight may be influenced by the presence of ANA Ab, and future studies to determine the underlying mechanism of this observation are warranted. Larger sample sizes may be needed to determine if there is a significant connection between neonatal head circumference and the presence of Anti- Brain Ab since there was a trend toward significance.

In the current study, a limitation was that Generation ECHO's data was restricted to mothers who… ELISA test (ANA). There was a lack of enriching for autoimmunity; the Generation ECHO project included data on autoimmunity but it was not utilized in this investigation. Another limitation was that the data from Generation ECHO had inevitable circumstances which did not allow for a thorough data collection. For example, inability to collect blood samples or having poor blood sample quality prevented ANA and anti-brain Ab tests from being performed; there was thus less data to determine results from.

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