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13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have identified 21,772 Child Health and Development Studies grandchildren (F2) by linking to the California Birth Records, which was slightly greater than our original estimate of 20,000. Using address history files, we verified 18,371 CHDS grandchildren births. By linking the verified births to the California Department of Developmental Services records we have successfully identified 116 autism cases in our cohort which exceeds our initial estimate of 72. We found Grandpaternal smoking was related to ASD (adjusted RR=2.03, 95% confidence interval [1.25, 3.3]), but grandmaternal smoking was not (aRR=0.9 [0.6, 1.5]). Increasing total alcohol consumption was suggestive of increased ASD risk (aRR=1.04 [0.96, 1.13] per grandmaternal drink consumed; aRR=1.04 [0.99, 1.10] per grandpaternal drink consumed), with stronger associations for hard alcohol (aRR=1.11 [1.00, 1.24] per grandpaternal drink consumed; aRR=1.15 [0.97, 1.36] per grandmaternal drink consumed). There was no association of ASD with increasing wine or beer consumption, or with any vs. no alcohol consumption, for either grandparent. Adjusting for F1 covariates including growth and development did not alter results. This is the first study of its kind in the United States, linking three generations studying germline exposures and risk of autism.

15. SUBJECT TERMS
Autism, prospective study, germline exposures, multi-generational cohort, grandparental risk factors

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1. INTRODUCTION:

This study will test the hypothesis that grandparental exposures during peri-conception and pregnancy predict increased risk of autism in the grandchildren. This study will identify cases of autism spectrum disorder (ASD) and unaffected controls in the grandchildren of The Child Health and Development Studies (CHDS) multigenerational cohort. We will use a prospective study of 18,317 verified CHDS grandchildren (F2) with 116 autism cases to explore the effect of grandparental (F0) exposures. The CHDS study population is a 50+ year follow-up of 20,020 pregnancies that occurred in the 1960s. Significantly, the 1960s was a period when maternal pregnancy exposures to a wide variety of endocrine active compounds were high, including prescription drugs, cigarette smoking, alcohol and coffee. We identified CHDS grandchildren by linking to California birth records. We identified grandchildren with autism by linking to California Department of Developmental Services (CA-DDS) files. Risk factors include grandmaternal and grandpaternal age, smoking, alcohol, coffee, and grandmaternal prescription drugs (tranquilizers, sedatives, amphetamines, diuretics, antihistamines hormones) during pregnancy.

2. KEYWORDS:

Autism, Prospective Study, Germline Exposures, Multi-generation Cohort, Grand-parental Risk Factors

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Submit for Local IRB (PHI) & CPHS approval. COMPLETED
2. Submit for DDS and Vital Records approval. COMPLETED
3. Submit for HRPO approvals. COMPLETED
4. Perform CHDS linkage to California Vital Statistics Birth Records to identify CHDS grandchild births. COMPLETED
5. Link CHDS grandchild birth records to the Department of Developmental Services records to identify cases of autism in grandchildren. COMPLETED
6. Link archive CHDS data on grandparents and parents to data generated on grandchildren. COMPLETED
7. Analysis of grandparental peri-conceptual and prenatal risk factors for grandchild autism. COMPLETED
8. Investigate relation of grandparental risk factors for autism to growth and development in the parent. COMPLETED

What was accomplished under these goals?

1. We have submitted and received approval from both Local IRB (Public Health Institute) & California Committee of Human Subjects (CPHS) approval.

2. We have submitted and received approval from the California Department of Health Information and Research Section to receive access to birth files from 1975 to 2014.
3. We have submitted and received approval from HRPO.
4. We have received the physical files containing the birth records from 1975 to 2014.
    a. We matched our cohort members (F1) to the California birth records and have completed this process.
    b. During the process we develop and refined our matching protocol and methods.
    c. We identified 21,772 F2, which was slightly greater than our original estimate of 20,000.
5. We had identified DDS variables we needed for the match and for analysis. We applied and received DDS approval and we have successfully run our CHDS state record file numbers against DDS records. By matching state record numbers (F2) to Department of Developmental Services we have successfully identified 131 autism cases in our cohort which exceeds our initial estimate of 72. Using past address history and other CHDS data we were able to verify 18,317 births and 116 autism causes in the CHDS F2 generation.
6. Using the 18,317 verified F2 identified births which contain the 116 autism cases we have created a data set that contains archived CHDS data on grandparents (F0) and parents (F1). We also appended the DDS data and variables available for the 116 identified autism cases.
7. We have evaluated the following grandmaternal and grandpaternal perinatal risk factors in relation to ASD: F0 age at delivery, smoking, alcohol consumption overall and by type, and caffeinated beverage consumption. We modeled the relative risk in binomial models with generalized estimating equations to account for clustering of grandchildren to the same grandparents. Covariates included age (when not the main risk factor), race, parity, grandpaternal education, and household income. Main findings:
    a. As expected from the literature, F1 age at birth of F2 was associated with ASD risk, however there was no evidence of increasing ASD risk with increasing grandmaternal or grandpaternal age at the time of the pregnancy (OR=0.95 [0.70, 1.28] and 1.02 [0.77, 1.36], respectively).
    b. As shown in Table 1, no associations were found for F0 maternal smoking. However, both categorical (yes/no) and continuous F0 smoking were associated with increased ASD risk. The greatest risk was for those who smoked greater than 20 cigarettes per day, and who started smoking as adults.
    c. As shown in Table 2, risk of ASD increased with the number of alcoholic beverages consumed by the mother or father, although the finding was of borderline statistical significance. In analysis by alcohol type, amount of whiskey was associated with increased ASD risk, but not amount of beer or wine consumption.
    d. We did not find any significant associations for either F0 male or F0 female caffeinated beverage consumption (tea and coffee combined), defined as binary or continuous, or specifically for tea and coffee separately (data not shown).

Findings from (a) and (b) above were published as an abstract by the International Society for Autism Research as part of their 2020 Virtual Meeting.

Table 1. Relative risk (RR)[a] of Autism Spectrum Disorder (ASD) associated with F0 maternal and paternal smoking

	Non-Case	Case	RR	95% CI
A. F0 Maternal Smoking				
Smoker (Yes vs No)	13904	85	0.92	(0.58, 1.48)
Number of cigarettes per day - continuous	13817	84	1.02	(0.91, 1.15)
1-9 cigarettes per day			0.78	(0.40, 1.51)
10-19 cigarettes per day	13817	84	1.35	(0.71, 2.56)
20+ cigarettes per day			1.03	(0.54, 1.99)
Age started smoking				
<=17 years old	12192	72	1.23	(0.58, 2.59)
18+ years old			0.76	(0.40, 1.43)
B. F0 Paternal Smoking				
Cigarette/cigar/pipe smoker	11921	76	**1.74**	**(1.04, 2.92)**
Cigarette smoker	11921	76	**1.63**	**(0.99, 2.69)**
Number of cigarettes per day - continuous	11371	72	**1.08**	**(0.98, 1.19)**
1-9 cigarettes per day			1.46	(0.66, 3.26)
10-19 cigarettes per day	11371	72	1.53	(0.64, 3.66)
20+ cigarettes per day			**1.81**	**(1.01, 3.25)**
Age started smoking				
<=17 years old	7463	46	1.58	(0.72, 3.45)
18+ years old			**2.38**	**(1.15, 4.91)**

[a]GEE models include clustering on F0, adjusted for: F0 parity, F0 maternal age, F0 family income, F0 paternal education, F0 maternal race, and either F0 maternal or F0 paternal drinking categories.

Table 2. Relative risk[a] of Autism Spectrum Disorder (ASD) associated with F0 maternal and paternal alcohol consumption

	Non-Case	Case	RR	95% CI
A. F0 Maternal alcohol consumption				
Any alcohol	12457	71	1.22	(0.71, 2.11)
Servings of alcohol per week - continuous	12457	71	**1.04**	**(0.96, 1.13)**
F0 mom alcoholic drinks per week - 1,2 (vs. 0)			1.11	(0.61, 2.03)
F0 mom alcoholic drinks per week - 3,4,5 (vs. 0)	13904	85	0.80	(0.26, 2.47)
F0 mom alcoholic drinks per week - 6+ (vs. 0)			1.12	(0.47, 2.71)
20+ alcoholic drinks per week (vs. fewer)	12457	71	0.99	(0.43, 2.31)
Servings of beer per week - continuous	12428	71	1.02	(0.88, 1.19)
Servings of wine per week - continuous	12409	70	1.00	(0.85, 1.17)
Servings of whisky per week	12389	70	**1.15**	**(0.97, 1.36)**

B. F0 Paternal alcohol consumption				
Any alcohol	10425	62	0.97	(0.53, 1.79)
Servings of alcohol per week - continuous	10425	62	**1.04**	**(0.99, 1.1)**
F0 mom alcoholic drinks per week - 1,2 (vs. 0)			0.74	(0.35, 1.57)
F0 mom alcoholic drinks per week - 3,4,5 (vs. 0)	11921	76	1.36	(0.66, 2.82)
F0 mom alcoholic drinks per week - 6+ (vs. 0)			1.00	(0.51, 1.96)
20+ alcoholic drinks per week (vs. fewer)	10425	62	1.15	(0.66, 1.99)
Servings of beer per week - continuous	10204	61	1.02	(0.94, 1.11)
Servings of wine per week - continuous	10195	60	1.06	(0.92, 1.21)
Servings of whisky per week	9973	60	**1.11**	**(1.00 1.24)**

[a]Adjusted for: F0 parity, F0 maternal age, F0 family income, F0 paternal education, F0 maternal race, and either F0 maternal or F0 paternal smoking status.

8. We have evaluated if measures of growth in the F1 influences any of the grandmaternal and grandpaternal perinatal risk factors in relation to ASD discussed above. We modeled the relative risk in binomial models with generalized estimating equations to account for clustering of grandchildren to the same grandparents. Covariates included age, race, parity, grandpaternal education and household income. F1 fetal growth did not change the granparental associations with F2 autism. We plan to submit results of these analysis to future conferences.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

We have discussed this project at our quarterly Participant Advisory Council (PAC) meetings. Our PAC is a diverse group of CHDS mothers, sons, and daughters who have partnered with us to help guide our research. They have expressed interest in studying Autism on multiple occasions and are eager to hear updates on our progress and findings in this study. We have reported and discussed our success with linking to the birth files. Our PAC is excited for the potential of this data and linkage for autism research, appreciates the increased scientific potential this linkage has built for the CHDS cohort.

We published findings as an abstract in the International Society for Autism Research 2020 Virtual Meeting.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Applying for and being granted IRB approval and linkage approval at Institutional, State and Federal levels, for linkage to public records sets a precedent for future linkages. This is an expansion of the permissions and linkages the CHDS already routinely conducts (DMV, CA death and CA cancer). Proving the feasibility and process of linking grand-parental health to grandchild health information will advance multigenerational and transgenerational research possibilities.

We are creating a matching procedure that CHDS and other researchers can use in conducting data linkages, especially for matching to California Vital Statistics data (California Birth Record Files). Briefly, birth record data available for matching in California has changed and expanded over the years requiring year-specific matching protocols. For example, before 1989, birthdate for mothers and fathers, and father's first name were not included in the electronic birth record files. This made the matches less reliable than matches done after 1989 where those variables were available to improve the matching integrity. We were able to support our matching results by using self-reported child bearing (births of F2) data collected from a subset of F1 women in our cohort. This comparison supported the validity of our matching to identify F2 births in our cohort. When comparing F1 women who previously self-reported having a child (n=2,029), 91% matched to the California Birth Record Files as mothers of an identified CHDS F2s. We would not expect a 100% match because not women who self-reported having children resided in California and our birth match was limited to California births.

By linking to our F2 we now have the possibility to expand our multigenerational and transgenerational research. We now have the link (state file record number) that will enable us to identify and obtain blood spots for our cohort offspring that were collected and archived by the state of California.

In this reporting year, we have successfully received approval from California Biobank Program (CBP) to receive blood spots for another study that will also look at autism. We will use the results from the match described above to choose and order blood spots from the California These blood spots will allow for environmental analysis of contaminants, genetic, and epigenetic markers in relation to autism risk. We will build on our analysis and findings presented above to examine autism risk factors in multiple generations.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

The COVID-19 pandemic caused the cancelation of conference where we had already and planned to submit abstracts and presentations. International Society for Autism Research 2020 meeting, where we had an accepted poster presentation, was switched to a virtual meeting. Unfortunately, this resulted in had reduced attendance and engagement. We plan to submit to conferences that will meet in 2021 or later, when we hope travel and in person meetings will be allowed.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
    - **Journal publications.**

      Nothing to report

- **Books or other non-periodical, one-time publications.**

  M. Pearl, K. Berger, N. Y. Krigbaum, P. M. Cirillo, V. Poon, B. Cohn and G. C. Windham; Grandparental Alcohol and Tobacco Use and Risk of Autism Spectrum Disorder in Grandchildren [Abstract]; International Society for Autism Research 2020 Virtual Meeting; https://insar.confex.com/insar/2020/meetingapp.cgi/Paper/35320

- **Other publications, conference papers, and presentations.**

  Nothing to report

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

  Nothing to report

- **Inventions, patent applications, and/or licenses**

  Nothing to report

- **Other Products**

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Barbara Cohn – No change

Nickilou Krigbaum- No change

Gayle Windham – No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

The following support began September 1, 2019

NIH 1R01MH118545 (Murphy)	09/01/19 – 08/31/2024
Barbara A. Cohn, co-investigator	1.2 calendar
NIH	
Early Life Exposures and Risk of Young-Onset Colorectal Cancer	

The following support began March 1, 2020
W81XWH-19-BCRP-BTA12 (Cohn and Jones) 03/01/20 – 02/28/23
Barbara Cohn, co-PI 1.2 calendar
Dept of the Army
Finding Metabolomic Signatures in Pregnancy that Predict Breast Cancer: 60-Year Prospective Study in the Child Health and Development Studies Pregnancy Cohort

What other organizations were involved as partners?

Nothing to report

5. SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS:

QUAD CHARTS:

6. APPENDICES: