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Grandmother's Race/Ethnicity, Grandparents' Age, and Grandmother's Pregnancy Complications on Autism Spectrum Disorders in Grandchildren, a California Multigenerational Birth Study

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Epidemiology

by

Ting Chow

2024

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ABSTRACT OF THE DISSERTATION

Grandmother's Race/Ethnicity, Grandparents' Age, and Grandmother's Pregnancy Complications on Autism Spectrum Disorders in Grandchildren, a California Multigenerational Birth Study

by

Ting Chow

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2024

Professor Beate R. Ritz, Chair

Autism spectrum disorders (ASD) is a neurodevelopmental disorder often characterized by difficulties in social communication and interaction, and by repetitive behaviors and restricted interests with symptoms starting in early childhood. ASD is multifactorial with risk factors including genetic, environmental, and demographic risk factors, and mother's pregnancy health. There has also been increasing evidence for multigenerational disease transmission of ASD in large European population-based studies. This dissertation investigated the associations between multigenerational risk factors and ASD in the ethnically and racially diverse California birth cohort.

The first study investigated grandmother's race, ethnicity, and nativity and ASD risk in grandchildren. The odds of ASD in grandchildren were higher among grandmothers who were US-born and foreign-born Black and Hispanic grandmothers compared to US-born White grandmothers.

The second study investigated grandmother and grandfather's age at the birth of their daughter with ASD risk in grandchildren and if these associations differ by race/ethnicity. The odds of ASD were elevated among younger (18-24 years) and older (35-55 years) maternal grandparents compared to grandparents of the reference age group (25-29 years). When we stratified by race/ethnicity, we found that the odds of ASD were higher among younger grandparents only among grandparents who were White. The odds of ASD were higher among older Hispanic grandmothers, Hispanic grandfathers, and Black grandfathers compared to the reference age group. The odds of ASD were also in the positive direction for older White grandparents, but the confidence intervals contained the null.

The third study investigated grandmother's pregnancy complications when pregnant with her daughter and ASD risk in grandchildren. The odds of ASD in grandchildren were higher among grandmothers who had hypertensive disorders or infections in pregnancy than grandmothers who did not have the respective conditions.

In conclusion, this dissertation corroborates previous findings of younger and older maternal grandparent's age and ASD. In addition, this dissertation investigated the associations between race, ethnicity, and nativity and ASD, as well as grandmother's hypertensive disorders and infection in pregnancy and ASD. These findings add to the current evidence of multigenerational disease transmission of ASD; suboptimal pregnancy conditions when the grandmother is pregnant with the mother's generation may impact neurodevelopment in grandchildren.

The dissertation of Ting Chow is approved.

Zeyan Liew

Roch Arnaud Kibsa Nianogo

May-Choo Wang

Beate R. Ritz, Committee Chair

University of California, Los Angeles

2024

To my brother, who took on responsibilities at an early age so we could be free to pursue our dreams.

Thank you for your support.

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ABBREVIATIONS AND ACRONYMS

ADHD	Attention-Deficit/Hyperactivity Disorder
APA	American Psychiatric Association
API	Asian Pacific Islander
ASD	Autism Spectrum Disorders
CA	California
CDC	Centers for Disease Control and Prevention
CDE	Controlled Direct Effect
CDER	Client Developmental Evaluation Report
CDPH	California Department of Public Health
DDS	Department of Developmental Services
DSM	Diagnostic and Statistical Manual of Mental Disorders
ICD	International Classification of Disease
ID	Intellectual Disabilities
LBW	Low Birth Weight
OR	Odds Ratio
PDD-NOS	Pervasive Developmental Disorder Not Otherwise Specified
PE	Portion Eliminated
PTB	Preterm Birth
TE	Total Effect
US	United States

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VITA

Education:

2017 MPH, University of California Los Angeles, Los Angeles, California.

2015 BS, University of California San Diego, La Jolla, California.

Publications:

Chow T, Meng Q, O'Sharkey K, Liew Z, Ritz B. Autism Spectrum Disorder and Grandmother's Race, Ethnicity, and Nativity. JAACAP Open. Epub 2024 Jun 7. doi: 10.1016/j.jaacop.2024.04.008.

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1. Background and Introduction

1.1 Autism Spectrum Disorders

1.1.1. Definition and Diagnostic Criteria

Autism Spectrum Disorders (ASD) is a neurodevelopmental disorder characterized by difficulty engaging in social interaction, repetitive behaviors, and restricted interests with symptoms presenting in early childhood [2,3]. ASD is diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) by the American Psychiatric Association (APA). In 2013, the APA introduced the DSM-V which defined ASD as one disorder without distinguishing between subcategories and a different set of diagnostic requirements [2,4]. According to the DSM-IV, the following pervasive developmental disorders are now what is considered ASD under the DSM-V: autistic disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS) [4,5].

The DSM-V was implemented in 2014 by the California Department of Developmental Services (DDS) [6]. In California, the DDS provides services and support to Californians with developmental disabilities including cerebral palsy, intellectual disability, down syndrome, autism, epilepsy and other related conditions [7,8]. The California DDS provides a variety of services to fit the needs of all individuals within their communities and have regional centers that provide these services throughout California [9,10]. According to the DDS best guideline practices, only professionals meeting strict qualifications may make a diagnosis of autism [11]. ASD assessments are made by interdisciplinary teams using the following six components: (1) review of relevant background information, (2) parent/caregiver interview, (3) comprehensive medical evaluation, (4) direct observation, (5) cognitive assessment, and (6) measures of adaptive function [11].

ASD often co-occurs with other developmental disabilities such as intellectual disability (ID), attention-deficit/hyperactivity disorder (ADHD), and epilepsy [2,12]. It has been estimated

that approximately 32% of children with ASD also have intellectual disability (intelligence quotient of 70 or less) [13,14].

1.1.2. Prevalence

The prevalence of ASD has been increasing over the past decades especially in California [15,16]. In 2020, the estimated prevalence of ASD among children 8-years of age was 27.6 per 1000 in the United States (US) and 44.9 per 1000 in California as reported by the US Centers of Disease Control and Prevention's (CDC) Autism and Developmental Disabilities Monitoring (ADDM) network [15].

1.1.3. Demographic Risk Factors

Demographic risk factors for ASD include birth year, sex, race/ethnicity, mother's nativity, and mother's age at delivery. Birth year and sex are strong risk factors of ASD. The prevalence of ASD has been increasing over the past decade [17]. The estimated prevalence of ASD in 2020 among 8-year-old children was also 3.8 times higher among boys than among girls [15].

Prior to 2014, the risk of ASD was estimated to be highest among children of White race, but lower among children who were Asian Pacific Islander (API), Black, and Hispanic [16]. However, in recent years, the prevalence of ASD is higher among children who are API, Black, and Hispanic [15,16,18]. This reversal in ASD prevalence by race and ethnicity may be from the change in the diagnostic practice of using the label "mental retardation" for children with ASD with intellectual disabilities (ID) [19] and/or a reduction of diagnosis disparities and reduction in barriers to health care access for children of minority backgrounds with less severe ASD [20]. Children from immigrant and refugee backgrounds are also at increased risk of ASD (including autism with comorbid ID) [21,22]. Differences in ASD (with or without ID) risk by race, ethnicity, and nativity have been hypothesized to be due to differences in adverse pregnancy environments such as environmental stressors [21-23] which are also more common in

communities of minority background [24,25]. ASD risk also increases with older maternal and paternal age, which is thought to be due to lowered quality of germ cells with age [26,27].

1.1.4. Health Risk Factors

Additional risk factors for ASD include the birth characteristics of the offspring as well as the mother's health when pregnant (the offspring in-utero environment). Offspring birth characteristic such as preterm birth (PTB) and/or low birth weight (LBW) are also risk factors for ASD [28].The in-utero environment when the mother is pregnant with the child may also influence offspring ASD risk. Epidemiologic studies have shown that hypertensive disorders [29,30], infections [31], and diabetes [32] in pregnancy increases the risk of ASD in offspring. Maternal hypertensive disorders and diabetes are thought to lead to placental disfunction, fetal hypoxia, and/or increased oxidative stress [30,32]. Infections in pregnancy may affect offspring neurodevelopment through actions of the pathogen themselves or triggering a harmful immune response of the mother to the pathogen [31].

1.2 Multigenerational Disease Transmission

1.2.1. Epidemiologic Studies

There is increasing evidence for multigenerational transmission of ASD. That is, exposures that occur when the grandmother is pregnant with the parental generation may influence the ASD risk in her grandchildren. Epidemiologic studies have found that grandparent's older and younger age [33-35], grandmother's smoking [36], and parental adverse birth characteristics [37] are associated with higher ASD risk in grandchildren. However, these studies are conducted in populations of predominately European ancestry of Denmark, Sweden, and United Kingdom.

1.2.2. Proposed Mechanisms

A potential mechanism for multigenerational disease transmission is germ-cell reprogramming through the parental germ cell line, where epigenetic marks typically removed and re-established in fetal development is disrupted [38-42]. Other proposed mechanisms for multigenerational disease transmission include cumulative mitochondrial DNA (mtDNA) damage during in-utero oocyte development for matrilineal transmission [43,44] or de novo mutations or epigenetic programming of the sperm transmitted patrilineally [45]. In human and/or animal studies, environmental exposures [46], stress [38], famine [47], and maternal immune activation [41] may have multigenerational impacts on health through these proposed mechanisms.

1.3 Research Gaps and Study Objectives

While there have been epidemiologic studies investigating some multigenerational risk factors of ASD, these studies are based on European study populations of predominantly White race [33-37]. There are also no current epidemiologic studies investigating grandmother's pregnancy complications and ASD risk in grandchildren. This dissertation investigated (1) whether grandmother's race, ethnicity, and nativity is associated with ASD risk in grandchildren, (2) whether race/ethnicity modifies the associations between maternal grandparents' younger and older age with ASD risk in grandchildren, and (3) whether grandmother's pregnancy complications when pregnant with her daughter is associated with ASD risk in grandchildren.

2. Autism Spectrum Disorders and Grandmother's Race, Ethnicity, and Nativity

2.1. Introduction

Autism spectrum disorder (ASD) is characterized by difficulty engaging in social interaction, repetitive behaviors, and restricted interests with symptoms presenting in early childhood [2,3]. The prevalence of ASD has been increasing over the past decades especially in California [15,16]. In 2020, the estimated prevalence of ASD among children 8-years of age was 27.6 per 1000 in the United States (US) and 44.9 per 1000 in California as reported by the US Centers of Disease Control and Prevention's (CDC) Autism and Developmental Disabilities Monitoring (ADDM) network [15].

Interestingly, while the reported prevalence of ASD was lower among children who were Asian Pacific Islander (API), Black, and Hispanic compared to children who were White prior to 2014 [16], more recently it is higher among children who were API, Black, and Hispanic [15,16,18]. This change in prevalence by race and ethnicity may be attributable to a reduction of disparities in diagnosis and barriers to accessing health care for children with less severe ASD in minorities [20] and/or a change in the diagnostic practice of using solely the label "mental retardation" for children with ASD with intellectual disabilities (ID) [19]. There is also growing evidence that children from immigrant and refugee backgrounds are at increased risk of ASD, including autism with comorbid ID [21,22]. It has been hypothesized that differences in ASD with/out ID risk by race, ethnicity, and nativity are due to differences in adverse pregnancy environments including environmental stressors [21-23] which are also more common in minority communities [24,25].

Adverse pregnancy environments might even increase ASD risk multigenerationally if they affect fetal germ cells, as some studies suggested happens in neurodevelopmental disorders including ASD [33-37], attention-deficit/hyperactivity disorder [48], and schizophrenia [49]. Epigenetic reprogramming occurs early in human embryonic development and is vulnerable to genetic and

environmental influences. This includes the fetal germ cells of the maternal generation that eventually develop into the second offspring generation [50-52]. Thus, multigenerational transmission of disease, while often considered genetic, might also be due to environmental factors that affect multiple generations, unless such exposures persist to affect the pregnancy of both the mother and grandmother. Nativity (immigration) changes the environment but not the genes of the grandmother, providing an opportunity to discern the influence of new exposures encountered due to immigration apart from genetic factors that act independent of the environment.

Epidemiologic studies investigating multigenerational risk factors for ASD are currently limited to populations of European-ancestry and predominantly White race [33-37]. Here, we investigate offspring ASD with/out co-occurring ID in the entire ethnically diverse California population. Children of immigrant and refugee backgrounds and of Black and Hispanic race and ethnicity regardless of nativity have been reported to have higher risk of ASD. Our study objective is to investigate associations between grandmother's race, ethnicity, and nativity and ASD in her grandchildren. We hypothesize that not just the children but also the grandchildren of women who are API, Black, and Hispanic have higher risks of developing ASD compared to grandchildren of grandmothers who were US-born White and that these risks may also further increase for grandchildren of grandmothers who were foreign-born.

2.2. Methods

Study Population

This study is a multigenerational California population-based birth cohort study of children born between 2001-2019 whose mothers were also born in California between 1983-2001. California birth records were obtained from the California Department of Public Health (CDPH) [53] which became electronically available in 1983. ASD diagnostic records were obtained from the

California Department of Developmental Services (DDS) [7]. According to DDS best practice guidelines, professionals must meet strict qualifications to make a diagnosis of autism, including California state licensure in the medical or mental health field. ASD assessments are made by well-trained interdisciplinary teams and include six components: (1) review of relevant background information, (2) parent/caregiver interview, (3) comprehensive medical evaluation, (4) direct observation, (5) cognitive assessment, and (6) measures of adaptive function [11]. ASD cases include individuals with a primary diagnosis of autistic disorder according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised (DSM-IV-R) until 2014 or DSM-V criteria as reported on the DDS Client Developmental Evaluation Report (CDER) [2,6,54]. These CDER records also include co-occurring ID coded with ICD-9 codes 317 (mild), 318.0 (moderate), 318.1 (severe), 318.2 (profound), 319 (unspecified) or ICD-10 codes F70 (mild), F71 (moderate), F72 (severe), F73 (profound), F78 (other), F79 (unspecified).

We first linked 2002-2022 ASD diagnostic records to 2001-2019 California birth records using probabilistic linkage (National Program of Cancer Registries Registry Plus Link Plus Software [55]) using similar methods previously described by other studies [56], based on child and parental identifiers (Table 2-S1). We were able to link 84.4% of the ASD cases to a California birth record. For this California birth cohort linked to DDS diagnostic records, we considered those not identified via linkage as non-cases (Figure 2-S1). Then starting with 2,371,799 births (2001-2019) for whom the birth certificate indicated that the mother was born in California from 1983 onwards (with non-missing maternal identifiers), we attempted to link the child to the mother's birth record using a similar linkage method as described above and succeeded for 2,162,154 (91.2%) births (Figure 2-S2). All California born offspring and mothers were eligible for this study if they had been singleton births with plausible birthweight (500-6,800 grams), gestational age (20-50 weeks), mother's and grandmother's ages were between 18-55 years, and information on child's sex, grandmother's parity, race, ethnicity, and nativity were available.

Our final analytical study population included 1,743,177 mother-child pairs with complete information for analyses (Figure 2-S3).

We used grandmother's self-reported race, Hispanic origin, and birthplace recorded on the mother's birth record and categorized race and ethnicity as API, Black, Hispanic, and White. Hispanic grandmothers are Hispanic of any race. We further split these groups into foreign-born and US-born (50 states and Washington D.C.). Grandmothers who were API were further split when sample size allowed, and grandmothers who were Hispanic were further split into Mexican and other Hispanic grandmothers.

Statistical Analysis

We performed logistic regression analyses to obtain odds ratios (ORs) and 95% CIs for ASD with/out co-occurring ID adjusting for child's birth year and sex (male, female participants), mother's birth year, grandmother's age at mother's delivery (18-24, 25-29, 30-34, 35-55 years), and grandmother's parity (1, 2, 3 or more births). We also additionally adjusted for grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more) as a sensitivity analysis for births beginning in 1989 when this data became first available. All covariates were recorded on the grandchild's or mother's birth certificates and included into regression models as categorical (see above) or continuous (birth year). Grandmothers who were US-born White were selected as a common reference group since recent ASD trends reported by the CDC's ADDM show lower ASD prevalence among children born 2010 and after who were White. We restricted some analyses to firstborn grandchildren, to address outcome correlatedness within families. Additionally, we conducted some analyses including mothers and grandmothers <18 and >55 years of age, to see if results change after including these less common but more complicated pregnancies.

Data analysis was performed using SAS 9.4 (SAS Institute Inc.). This study has been approved by the California State and University of California Los Angeles institutional review boards.

2.3. Results

In our study population of 1,743,177 children, 27,965 (1.6%) were diagnosed with ASD and 3,385 (0.2%) with ASD and ID (Table 2-1, Table 2-2). While the birth cohort consisted of 51.1% male offspring, cases consisted of 78.5% male offspring. Mothers of these children were born between 1983-2001 (Table 2-S2) and 31.8% were the firstborn child of the grandmother. Grandmothers were on average 25.9 years old at the mother's time of birth and most grandmothers had given birth to their daughters by the age of 30 years.

The odds of offspring ASD differed slightly by grandmother's race, ethnicity, and nativity after adjusting for child's birth year, child's sex, mother's birth year, grandmother's parity, and grandmother's age (Table 2-3). The odds of ASD in the grandchildren were higher among grandmothers who were US-born Black (OR 1.25, 95% CI 1.20-1.31) and US-born Hispanic (OR 1.19, 95% CI 1.14-1.23), and increased further among grandmothers who were foreign-born Black (OR 1.45, 95% CI 1.17-1.81) but not foreign-born Hispanic (OR 1.22, 95% CI 1.19-1.26) compared to grandmother who were US-born White. Further subgroup analyses revealed that the odds of ASD in the grandchildren were higher among grandmothers who were foreign-born other Hispanic (OR 1.47, 95% CI 1.39-1.56) compared to grandmothers who were US-born White. The odds of ASD in the grandchildren were similar or slightly lower among grandmothers who were US-born API (OR 0.87, 95% CI 0.75-1.02), foreign-born API (OR 0.94, 95% CI 0.88-1.00) and foreign-born White (OR 0.90, 95% CI 0.82-0.99) compared to grandmothers who were US-born White as the confidence intervals included or were close to the null value of 1.

We generally estimated stronger effect estimates for the outcome ASD with co-occurring ID (Table 2-4). The odds of ASD with ID in grandchildren were again higher among grandmothers who were US-born Black (OR 1.84, 95% CI 1.62-2.08) and US-born Hispanic (OR 1.63, 95% CI 1.47-1.82), and the odds increased even further among grandmothers who were foreign-born Black (OR 2.97, 95% CI 1.81-4.88) but less among foreign-born Hispanic (OR 1.70, 95% CI 1.55-1.86) compared to grandmothers who were US-born White. In contrast, while there was now also an increase in the odds of ASD with ID among grandchildren of grandmothers who were foreign-born API (OR 1.42, 95% CI 1.19-1.69), there still were no differences observed for grandmothers who were US-born API (OR 1.08, 95% CI 0.69-1.71) or foreign-born White (OR 0.91, 95% CI 0.67-1.23) compared to grandmothers who were US-born White.

Restricting the study population to mothers born 1989-2001 to control for grandmother's education shifted the point estimates for offspring ASD and grandmother's race, ethnicity, and nativity towards the null. However, this shift was due to the change in the population analyzed as ORs estimated with and without adjustment for grandmother's education were quite similar (Table 2-S3). A similar pattern was observed for ASD with ID (Table 2-S4). Results also did not change more than minimally after restricting to firstborn grandchildren (Table 2-S5, available online). Results also remained similar without restricting mother and grandmother's ages to 18-55 years (Table 2-S6).

2.4. Discussion

We found that grandmother's race, ethnicity, and nativity influence the risk of ASD in her grandchildren. The odds of ASD with or without ID in the grandchildren were highest among grandmothers who were foreign-born Black and Hispanic compared to grandmothers who were US-born White. The effect estimates were also increased but less strongly for grandmothers who were US-born Black and Hispanic. Offspring ASD risks were generally similar or even

slightly lower among grandmothers who were US- and foreign-born API and foreign-born White compared to grandmothers who were US-born White.

For ASD with ID, all associations, except for grandmothers who were foreign-born White, were positive in direction and stronger in size possibly reflecting better ascertainment of cases among minority and immigrant populations since ASD with ID may lead to earlier referral and evaluation regardless of socioeconomic status or maternal education [57]. The higher odds of ASD with ID among grandmothers who were foreign-born API compared to US-born White may reflect an especially high prevalence among grandmothers who were foreign-born Filipino since they comprised about one third of the ASD with ID cases among grandmothers who were API.

The higher increased risks of ASD in grandchildren we observed for grandmothers who were foreign-born Black and Hispanic may represent differences in dietary factors (or deficiencies) [58-60] or adverse physical or social environments prior to their immigration (including stress from fleeing war-ridden nations [61-66]) or the environments they encountered after immigration (including living in areas with higher air pollution or social or cultural isolation [25,67-69]). In murine models, exposure to stress or conditioned fear when the grandmother is pregnant with the daughter has led to altered behavior in the daughter's offspring generation, potentially due to epigenetic germ cell reprogramming [38]. Associations between prenatal diet or famine with epigenetic alterations and with the health of subsequent generations have been reported in human populations and animal models [47]. The increased risk of ASD with ID among grandchildren we observed for grandmothers who were foreign-born Filipino, however, may instead represent the unique exposures of healthcare related work including infectious exposures, as most women who were Filipino of this generation came to California for healthcare related work [70]. In murine models, it has been shown that maternal immune

activation may have multigenerational impacts through epigenetic modifications and germ-cell dependent inheritance [41].

The stronger effect estimates by nativity suggest that either the immigration experience or similar environmental factors experienced in the host country rather than the experiences in the diverse countries of origin are relevant since grandmothers of the same race and ethnicity may share similar genetic variants but not environmental exposures before and after immigration. Nativity seemed to play a stronger role for ASD with than without ID in grandchildren among grandmothers who were API (US-born OR 1.08 vs foreign-born OR 1.42) and Black (US-born OR 1.84 vs foreign-born OR 2.97) perhaps related to unique stressors related to migration (Table 2-4). A previous Australian study reported that the risk of ASD with ID was higher among children of mothers from foreign-born low-income countries [71]. A Swedish study found that children of parents who immigrated had increased risk of autism presenting with comorbid ID and they attributed this to environmental stressors related to migration [22].

However, more research is needed to understand ASD with ID and migration stress generally and in the multigenerational context since ASD without ID may also simply be under-diagnosed and undertreated in immigrant population offspring. It is possible that underdiagnosis of ASD without ID is higher among children of foreign-born than US-born grandmothers who were API and Black. Perhaps with greater assimilation, some persons of color (API and Black) become more aware of the resources available in California for ASD without ID. However, our results that grandchildren of all grandmothers who were foreign-born (except for grandmothers who were White) were at a higher risk of ASD with/out ID than their US-born counterparts are intriguing as it suggests that the new environments they encountered in their host country may have contributed to this heightened risk. While studies have shown that some environmental stressors are more commonly found in minority neighborhoods [24,25], more research is

needed to understand whether minority migration may place immigrants into potentially disadvantaged or poorer neighborhoods with greater environmental challenges.

We conducted sensitivity analyses additionally adjusting for grandmother's education but acknowledge that grandmother's education may be a mediator rather than a confounder. Results remained quite similar with or without adjusting for grandmother's education, but the effect estimates became slightly weaker for grandmothers who were US-born Black and Hispanic and slightly stronger for grandmothers who were foreign-born Black and Hispanic compared to grandmothers who were US-born White (Table 2-S3). A similar pattern was observed for ASD with ID (Table 2-S4). The effect attenuation for grandmothers who were US-born Black and Hispanic may indicate that education plays a role in explaining some of the association between race and ethnicity and ASD in grandchildren among grandmothers born in the US. In our multigenerational study population, grandmothers who were foreign-born were generally less well-educated than grandmothers who were US-born of the same race and ethnicity (Table 2-S7). We additionally recognize that while age and parity are common covariates for adjustments in birth studies, grandmother's age and grandmother's parity may also be mediators on the path for the association between grandmother's race, ethnicity, and nativity with ASD in grandchildren. However, the results with minimal adjustment for grandchild's birth year and sex (strong risk factors for ASD) are very similar to those of the fully adjusted model.

Only in the last decade has the reported prevalence of ASD been lower among children who were White compared to children who were API, Black, and Hispanic i.e. for those born after 2010 [16]. We are presenting results for a multigenerational population of grandchildren born 2001-2019, thus including children born in the first decade of the 2000s attenuates our results when using grandmothers who were US-born White as a common reference group (Table 2-S8).

The stronger effect estimates for 2011-2019 births likely indicate better ASD ascertainment for these later birth years and support the hypotheses of our study more strongly.

A strength of this study is its relatively large size, its California wide coverage, and the fact that grandmother's race, ethnicity, and nativity could be obtained from birth records eliminating the possibility of recall or participation bias, as none of the women self-selected into our study and cases and non-cases were all enrolled according to the same eligibility criteria. While ASD case identification by the DDS has a high specificity (low false positive rate), the sensitivity may have varied over the years of our study as awareness of ASD – especially among minority and immigrant communities – has risen. We attempted to address this by adjusting for child's birth year in our covariate adjustment as well as assessing both ASD with/out co-occurring ID as our outcome and distinguishing between two birth year periods (2001-2010, 2011-2019).

Additional limitations to consider include the age truncation of the mother's generation (and the potential age truncation of the grandmother's generation) due to birth certificate data availability, which may have differed by race, ethnicity, and nativity. Comparing results for mother's race, ethnicity, and nativity in our restricted multigenerational study population to results in the full birth cohort, our results for the multigenerational study population were attenuated (Table 2-S9). This suggests a possible underestimation of the association strengths for grandmother's race, ethnicity, and nativity. Our sample size was too limited to investigate specific groups among grandmothers who were API except for grandmothers who were foreign-born Filipino. Like in any birth cohort study, there may be live birth bias which typically results in negative bias towards the null. Whether this also applies in the multigenerational context needs to be further explored.

Additionally, while our results are generalizable to California births, results might not be generalizable to other states due to differences in immigration or the availability of developmental services to all children. ASD risks may also vary according to other factors including geographic area. When we repeated our analyses by geographic region of California we did not find notable differences. However, this does not mean that differences do not exist as we may currently not have the statistical power to discern such differences. Further studies with longer follow-up are needed to re-examine this hypothesis.

2.5. Conclusion

Our findings suggest that grandchildren of grandmothers who were US- and foreign-born Black and Hispanic are at higher risk of developing ASD including ASD with ID than grandchildren of grandmothers who were US-born White. This suggests that there may be multigenerational risk factors for ASD in this racially and ethnically diverse population. While differences in ASD risk by race, ethnicity, and nativity have been hypothesized to be potentially due to differences in environmental factors, future studies with actual measures of multigenerational environmental stressors (such as air pollution) are needed.

2.6. Tables

Table 2-1. Study Population Characteristics by Autism Spectrum Disorder

Study Population Characteristics	**Case (n=27,965)**	**Total (N=1,743,177)**
Child's Sex, n (%)		
Female	6,014 (21.5)	852,561 (48.9)
Male	21,951 (78.5)	890,616 (51.1)
Mother's Birth Year, mean (SD)	1,988.6 (4.00)	1,988.2 (3.94)
Grandmother's Parity, n (%)		
1	9,089 (32.5)	553,994 (31.8)
2	8,429 (30.1)	539,319 (30.9)
3 or more	10,447 (37.4)	649,864 (37.3)
Grandmother's Age at Mother's Delivery, mean (SD), years	25.9 (5.42)	25.9 (5.31)
Grandmother's Age at Mother's Delivery, n (%), years		
18-24 years	12,651 (45.2)	783,280 (44.9)
25-29 years	8,377 (30.0)	535,098 (30.7)
30-34 years	4,735 (16.9)	298,046 (17.1)
35-55 years	2,202 (7.9)	126,753 (7.3)

Table 2-2. Study Population Characteristics by Autism Spectrum Disorder with Intellectual Disabilities

Study Population Characteristics	**Case (n=3,385)**	**Total (N=1,743,177)**
Child's Sex, n (%)		
Female	792 (23.4)	852,561 (48.9)
Male	2,593 (76.6)	890,616 (51.1)
Mother's Birth Year, mean (SD)	1,988.5 (4.04)	1,988.2 (3.94)
Grandmother's Parity, n (%)		
1	1,042 (30.8)	553,994 (31.8)
2	1,010 (29.8)	539,319 (30.9)
3 or more	1,333 (39.4)	649,864 (37.3)
Grandmother's Age at Mother's Delivery, mean (SD), years	25.9 (5.45)	25.9 (5.31)
Grandmother's Age at Mother's Delivery, n (%), years		
18-24 years	1,535 (45.3)	783,280 (44.9)
25-29 years	1,020 (30.1)	535,098 (30.7)
30-34 years	557 (16.5)	298,046 (17.1)
35-55 years	273 (8.1)	126,753 (7.3)

Table 2-3. Offspring Autism Spectrum Disorder and Grandmother's Race, Ethnicity, and Nativity

	Children, n		OR (95% CI)	
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 0** [a]	**Model 1** [b]
Asian Pacific Islander				
US-born	170	12,879	0.87 (0.75-1.02)	0.87 (0.75-1.02)
Foreign-born	1,132	79,677	0.94 (0.89-1.01)	0.94 (0.88-1.00)
Filipino	382	24,698	1.04 (0.93-1.15)	1.03 (0.93-1.14)
Black				
US-born	2,532	147,525	1.24 (1.19-1.30)	1.25 (1.20-1.31)
Foreign-born	82	4,003	1.45 (1.16-1.81)	1.45 (1.17-1.81)
Hispanic of any race				
US-born	4,793	288,421	1.18 (1.14-1.22)	1.19 (1.14-1.23)
Mexican	3,922	234,297	1.19 (1.14-1.23)	1.20 (1.15-1.24)
Other Hispanic	871	54,124	1.14 (1.06-1.22)	1.15 (1.07-1.23)
Foreign-born	10,419	598,663	1.21 (1.18-1.25)	1.22 (1.19-1.26)
Mexican	8,876	525,086	1.18 (1.14-1.21)	1.19 (1.15-1.23)
Other Hispanic	1,543	73,577	1.46 (1.38-1.54)	1.47 (1.39-1.56)
White				
US-born	8,354	576,563	Reference	Reference
Foreign-born	483	35,446	0.91 (0.83-1.00)	0.90 (0.82-0.99)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. OR = odds ratio.

[a] Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

[b] Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandmother's age (18-24, 25-29, 30-34, 35-55 years).

Table 2-4. Offspring Autism Spectrum Disorder with Intellectual Disabilities and Grandmother's Race, Ethnicity, and Nativity

	Children, n		OR (95% CI)	
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 0** [a]	**Model 1** [b]
Asian Pacific Islander				
US-born	19	12,879	1.08 (0.69-1.71)	1.08 (0.69-1.71)
Foreign-born	155	79,677	1.43 (1.20-1.70)	1.42 (1.19-1.69)
Filipino	49	24,698	1.46 (1.09-1.95)	1.45 (1.09-1.94)
Black				
US-born	362	147,525	1.84 (1.62-2.09)	1.84 (1.62-2.08)
Foreign-born	16	4,003	2.99 (1.82-4.90)	2.97 (1.81-4.88)
Hispanic of any race				
US-born	633	288,421	1.64 (1.47-1.82)	1.63 (1.47-1.82)
Mexican	516	234,297	1.64 (1.47-1.84)	1.64 (1.47-1.84)
Other Hispanic	117	54,124	1.61 (1.33-1.96)	1.61 (1.33-1.96)
Foreign-born	1,377	598,663	1.71 (1.57-1.87)	1.70 (1.55-1.86)
Mexican	1,202	525,086	1.70 (1.56-1.86)	1.69 (1.54-1.86)
Other Hispanic	175	73,577	1.76 (1.50-2.08)	1.75 (1.49-2.07)
White				
US-born	779	576,563	Reference	Reference
Foreign-born	44	35,446	0.91 (0.67-1.24)	0.91 (0.67-1.23)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. OR = odds ratio.

[a] Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

[b] Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandmother's age (18-24, 25-29, 30-34, 35-55 years).

2.7. Supplemental Materials

Table 2-S1. Variables for Probabilistic Linkage

DDS to Birth Linkage
Child's Date of Birth
Child's First Name
Child's Last Name
Child's Sex
Mother's Date of Birth
Mother's First Name
Mother's Surname
Father's Date of Birth
Father's First Name
Father's Last Name
Zipcode
Race
Hispanic Origin
Multigenerational Linkage
Mother's Date of Birth
Mother's First Name
Mother's Surname
Race
Hispanic Origin

Table 2-S2. Child's and Mother's Birth Years

Child's Birth Year, n (%)	**Total (N=1,743,177)**
2001	2,874 (0.2)
2002	10,021 (0.6)
2003	18,861 (1.1)
2004	28,233 (1.6)
2005	38,247 (2.2)
2006	50,797 (2.9)
2007	60,450 (3.5)
2008	74,907 (4.3)
2009	84,443 (4.8)
2010	94,265 (5.4)
2011	104,371 (6.0)
2012	116,210 (6.7)
2013	126,512 (7.3)
2014	138,541 (7.9)
2015	146,990 (8.4)
2016	155,757 (8.9)
2017	160,455 (9.2)

2018	162,809 (9.3)
2019	168,434 (9.7)
Mother's Birth Year, n (%)	**Total (N=1,743,177)**
1983	183,229 (10.5)
1984	176,537 (10.1)
1985	171,828 (9.9)
1986	160,827 (9.2)
1987	151,280 (8.7)
1988	144,868 (8.3)
1989	138,479 (7.9)
1990	130,997 (7.5)
1991	113,034 (6.5)
1992	94,391 (5.4)
1993	77,583 (4.5)
1994	62,128 (3.6)
1995	47,478 (2.7)
1996	35,456 (2.0)
1997	25,113 (1.4)
1998	15,566 (0.9)
1999	9,126 (0.5)
2000	4,204 (0.2)
2001	1,053 (0.1)

Table 2-S3. Offspring Autism Spectrum Disorder and Grandmother's Race, Ethnicity, and Nativity, among Mothers born 1989-2001 and non-missing Grandmother's Education

	Children, n		OR (95% CI)	
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]	**Model 2** [b]
Asian Pacific Islander				
US-born	64	4,525	0.83 (0.64-1.06)	0.83 (0.65-1.07)
Foreign-born	342	23,794	0.84 (0.75-0.94)	0.86 (0.77-0.97)
Filipino	107	7,193	0.86 (0.70-1.04)	0.89 (0.73-1.09)
Black				
US-born	1,165	65,339	1.11 (1.03-1.19)	1.09 (1.02-1.17)
Foreign-born	32	1,611	1.20 (0.84-1.71)	1.22 (0.85-1.73)
Hispanic of any race				
US-born	2,333	131,846	1.09 (1.03-1.15)	1.07 (1.01-1.13)
Mexican	2,005	111,690	1.10 (1.04-1.17)	1.08 (1.02-1.15)
Other Hispanic	328	20,156	1.02 (0.91-1.14)	1.00 (0.89-1.12)
Foreign-born	5,942	320,519	1.14 (1.09-1.19)	1.15 (1.09-1.21)
Mexican	5,131	284,404	1.11 (1.06-1.16)	1.12 (1.06-1.18)
Other Hispanic	811	36,115	1.38 (1.28-1.50)	1.39 (1.28-1.50)
White				
US-born	3,182	189,458	Reference	Reference
Foreign-born	156	10,749	0.84 (0.71-0.99)	0.85 (0.72-1.00)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. OR = odds ratio.

[a] Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female participants), mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandmother's age (18-24, 25-29, 30-34, 35-55 years).

[b] Model 2 adjusts for the variables in model 1 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more).

Table 2-S4. Offspring Autism Spectrum Disorder with Intellectual Disabilities and Grandmother's Race, Ethnicity, and Nativity, among Mothers born 1989-2001 and non-missing Grandmother's Education

	Children, n		OR (95% CI)	
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]	**Model 2** [b]
Asian Pacific Islander				
US-born	8	4,525	1.35 (0.67-2.72)	1.36 (0.67-2.75)
Foreign-born	41	23,794	1.31 (0.94-1.82)	1.35 (0.97-1.89)
Filipino	14	7,193	1.43 (0.84-2.46)	1.49 (0.87-2.56)
Black				
US-born	156	65,339	1.90 (1.55-2.33)	1.89 (1.54-2.31)
Foreign-born	10	1,611	4.80 (2.54-9.04)	4.87 (2.58-9.18)
Hispanic of any race				
US-born	293	131,846	1.78 (1.50-2.11)	1.73 (1.46-2.06)
Mexican	253	111,690	1.81 (1.52-2.17)	1.76 (1.47-2.11)
Other Hispanic	40	20,156	1.60 (1.14-2.24)	1.57 (1.12-2.20)
Foreign-born	778	320,519	1.91 (1.65-2.21)	1.95 (1.65-2.29)
Mexican	684	284,404	1.90 (1.64-2.20)	1.93 (1.64-2.29)
Other Hispanic	94	36,115	2.03 (1.60-2.57)	2.05 (1.60-2.62)
White				
US-born	247	189,458	Reference	Reference
Foreign-born	18	10,749	1.26 (0.78-2.03)	1.27 (0.79-2.05)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. OR = odds ratio.

[a] Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female participants), mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandmother's age (18-24, 25-29, 30-34, 35-55 years).

[b] Model 2 adjusts for the variables in model 1 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more).

Table 2-S5. Offspring Autism Spectrum Disorder and Grandmother's Race, Ethnicity, and Nativity, among Firstborn Offsprings

	Children, n		OR (95% CI)
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]
Asian Pacific Islander			
US-born	103	7,304	0.81 (0.66-0.98)
Foreign-born	694	43,788	0.90 (0.83-0.98)
Filipino	252	14,464	1.00 (0.88-1.14)
Black			
US-born	1,397	74,769	1.25 (1.18-1.33)
Foreign-born	52	2,279	1.48 (1.12-1.95)
Hispanic of any race			
US-born	2,435	136,126	1.15 (1.10-1.21)
Mexican	1,988	110,150	1.16 (1.10-1.23)
Other Hispanic	447	25,976	1.11 (1.01-1.23)
Foreign-born	5,446	292,610	1.19 (1.14-1.24)
Mexican	4,575	253,732	1.16 (1.11-1.20)
Other Hispanic	871	38,878	1.41 (1.31-1.52)
White			
US-born	5,004	305,699	Reference
Foreign-born	291	19,846	0.84 (0.75-0.95)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. OR = odds ratio.

[a] Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female participants), mother's birth year (continuously scaled), and grandmother's age (18-24, 25-29, 30-34, 35-55 years).

Table 2-S6. Offspring Autism Spectrum Disorder and ASD with Intellectual Disabilities and Grandmother's Race, Ethnicity, and Nativity, without Mother or Grandmother's Age 18-55 Years Restriction

	ASD			**ASD with ID**		
	Children, n		**OR (95% CI)**	**Children, n**		**OR (95% CI)**
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]	**Cases**	**Total**	**Model 1** [a]
Asian Pacific Islander						
US-born	193	14,392	0.90 (0.78-1.03)	21	14,392	1.06 (0.69-1.63)
Foreign-born	1,195	85,720	0.93 (0.87-0.99)	163	85,720	1.37 (1.15-1.62)
Filipino	395	25,919	1.02 (0.92-1.13)	53	25,919	1.47 (1.11-1.94)
Black						
US-born	2,932	179,014	1.24 (1.18-1.29)	427	179,014	1.81 (1.61-2.04)
Foreign-born	90	4,435	1.48 (1.20-1.83)	17	4,435	2.86 (1.77-4.63)
Hispanic of any race						
US-born	5,815	363,751	1.18 (1.14-1.22)	756	363,751	1.57 (1.42-1.74)
Mexican	4,798	298,237	1.19 (1.15-1.23)	611	298,237	1.55 (1.39-1.72)
Other Hispanic	1,017	65,514	1.15 (1.07-1.22)	145	65,514	1.67 (1.40-1.99)
Foreign-born	11,435	695,737	1.20 (1.17-1.24)	1,546	695,737	1.66 (1.52-1.81)
Mexican	9,744	611,652	1.16 (1.13-1.20)	1,350	611,652	1.65 (1.51-1.80)
Other Hispanic	1,691	84,085	1.46 (1.38-1.54)	196	84,085	1.73 (1.48-2.02)
White						
US-born	8,928	624,611	Reference	850	624,611	Reference
Foreign-born	504	36,992	0.90 (0.83-0.99)	53	36,992	1.03 (0.78-1.36)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. ASD = autism spectrum disorder; ID = intellectual disabilities; OR = odds ratio.

[a] Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female participants), mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandmother's age (<=17, 18-24, 25-29, 30-34, >=35 years).

Table 2-S7. Grandmother's Education Distribution by Grandmother's Race, Ethnicity, and Nativity

	Grandmother's Race, Ethnicity, and Nativity								
	US-born Asian Pacific Islander (n=4,525)	**US-born Black (n=65,339)**	**US-born Hispanic of any race (n=131,846)**	**US-born White (n=189,458)**	**foreign-born Asian Pacific Islander (n=23,794)**	**foreign-born Black (n=1,611)**	**foreign-born Hispanic of any race (n=320,519)**	**foreign-born White (n=10,749)**	**Total (N=747,841)**
Grandmother's Education, n (%)									
8th grade or less	46 (1.0)	588 (0.9)	4,578 (3.5)	2,651 (1.4)	7,987 (33.6)	78 (4.8)	165,576 (51.7)	1,266 (11.8)	182,770 (24.4)
9th to 12th grade (no diploma)	749 (16.6)	14,167 (21.7)	49,282 (37.4)	35,466 (18.7)	2,610 (11.0)	205 (12.7)	83,020 (25.9)	1,626 (15.1)	187,125 (25.0)
High School graduate	2,098 (46.4)	34,525 (52.8)	58,663 (44.5)	90,560 (47.8)	6,414 (27.0)	705 (43.8)	53,340 (16.6)	4,190 (39.0)	250,495 (33.5)
Some Degree less than College	1,114 (24.6)	13,896 (21.3)	16,919 (12.8)	44,266 (23.4)	4,200 (17.7)	447 (27.7)	14,992 (4.7)	2,361 (22.0)	98,195 (13.1)
College or More	518 (11.4)	2,163 (3.3)	2,404 (1.8)	16,515 (8.7)	2,583 (10.9)	176 (10.9)	3,591 (1.1)	1,306 (12.1)	29,256 (3.9)
Grandmother's Education, n (%)									
Less than High School	795 (17.6)	14,755 (22.6)	53,860 (40.9)	38,117 (20.1)	10,597 (44.5)	283 (17.6)	248,596 (77.6)	2,892 (26.9)	369,895 (49.5)
High School or more	3,730 (82.4)	50,584 (77.4)	77,986 (59.1)	151,341 (79.9)	13,197 (55.5)	1,328 (82.4)	71923 (22.4)	7,857 (73.1)	377,946 (50.5)
Grandmother's Education, n (%)									
High School or Less	2,893 (63.9)	49,280 (75.4)	112,523 (85.3)	128,677 (67.9)	17,011 (71.5)	988 (61.3)	301,936 (94.2)	7,082 (65.9)	620,390 (83.0)
More than High School	1,632 (36.1)	16,059 (24.6)	19,323 (14.7)	60,781 (32.1)	6,783 (28.5)	623 (38.7)	18,583 (5.8)	3,667 (34.1)	127,451 (17.0)

Table 2-S8. Offspring Autism Spectrum Disorder and ASD with Intellectual Disabilities and Grandmother's Race, Ethnicity, and Nativity, among Offspring born 2001-2010 and 2011-2019

	ASD			**ASD with ID**		
Offspring born 2001-2010	**Children, n**		**OR (95% CI)**	**Children, n**		**OR (95% CI)**
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]	**Cases**	**Total**	**Model 1** [a]
Asian Pacific Islander						
US-born	25	2,569	0.74 (0.50-1.11)	5	2,569	1.05 (0.43-2.54)
Foreign-born	147	15,969	0.70 (0.59-0.83)	35	15,969	1.16 (0.82-1.66)
Filipino	40	5,131	0.57 (0.41-0.78)	8	5,131	0.82 (0.40-1.66)
Black						
US-born	527	48,587	0.88 (0.80-0.97)	103	48,587	1.17 (0.93-1.47)
Foreign-born	15	1,145	1.03 (0.62-1.72)	3	1,145	1.42 (0.45-4.43)
Hispanic of any race						
US-born	934	85,955	0.89 (0.82-0.96)	168	85,955	1.07 (0.88-1.30)
Mexican	738	69,816	0.86 (0.79-0.94)	133	69,816	1.04 (0.84-1.28)
Other Hispanic	196	16,139	0.97 (0.84-1.13)	35	16,139	1.23 (0.86-1.75)
Foreign-born	1,571	154,683	0.81 (0.75-0.86)	261	154,683	0.92 (0.77-1.10)
Mexican	1,301	135,839	0.76 (0.71-0.82)	225	135,839	0.91 (0.76-1.09)
Other Hispanic	270	18,844	1.11 (0.98-1.27)	36	18,844	1.03 (0.73-1.47)
White						
US-born	1,909	147,140	Reference	274	147,140	Reference
Foreign-born	80	7,050	0.86 (0.69-1.08)	10	7,050	0.76 (0.41-1.43)
Offspring born 2011-2019	**Children, n**		**OR (95% CI)**	**Children, n**		**OR (95% CI)**
Grandmother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]	**Cases**	**Total**	**Model 1** [a]
Asian Pacific Islander						
US-born	145	10,310	0.92 (0.78-1.09)	14	10,310	1.15 (0.67-1.95)
Foreign-born	985	63,708	1.01 (0.95-1.08)	120	63,708	1.58 (1.30-1.93)
Filipino	342	19,567	1.15 (1.03-1.29)	41	19,567	1.76 (1.28-2.42)
Black						
US-born	2,005	98,938	1.38 (1.31-1.45)	259	98,938	2.24 (1.93-2.61)

Foreign-born	67	2,858	1.59 (1.24-2.03)	13	2,858	3.88 (2.23-6.74)
Hispanic of any race						
US-born	3,859	202,466	1.28 (1.23-1.34)	465	202,466	1.96 (1.73-2.24)
Mexican	3,184	164,481	1.31 (1.25-1.36)	383	164,481	1.99 (1.74-2.29)
Other Hispanic	675	37,985	1.20 (1.11-1.30)	82	37,985	1.86 (1.47-2.35)
Foreign-born	8,848	443,980	1.35 (1.31-1.40)	1116	443,980	2.14 (1.92-2.39)
Mexican	7,575	389,247	1.32 (1.28-1.37)	977	389,247	2.14 (1.92-2.39)
Other Hispanic	1,273	54,733	1.58 (1.49-1.68)	139	54,733	2.16 (1.79-2.61)
White						
US-born	6,445	429,423	Reference	505	429,423	Reference
Foreign-born	403	28,396	0.93 (0.84-1.03)	34	28,396	1.01 (0.71-1.43)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. ASD = autism spectrum disorder; ID = intellectual disabilities; OR = odds ratio.

[a] Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female participants), mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandmother's age (18-24, 25-29, 30-34, 35-55 years).

Table 2-S9. Offspring Autism Spectrum Disorder and Mother's Race, Ethnicity, and Nativity

	Multigenerational Study Population			Full 2001-2019 Birth Cohort		
	Children, n		OR (95% CI)	Children, n		OR (95% CI)
Mother's Race, Ethnicity, and Nativity	**Cases**	**Total**	**Model 1** [a]	**Cases**	**Total**	**Model 1** [a]
Asian Pacific Islander						
US-born	1,003	70,681	0.98 (0.92-1.05)	2,989	236,529	0.93 (0.90-0.97)
Foreign-born				13,551	967,568	1.04 (1.02-1.06)
Filipino				3,573	174,340	1.66 (1.60-1.72)
Black						
US-born	2,269	134,602	1.34 (1.27-1.40)	6,780	411,684	1.56 (1.52-1.61)
Foreign-born				1,062	46,641	1.83 (1.71-1.94)
Hispanic of any race						
US-born	15,782	921,023	1.31 (1.27-1.34)	32,165	2,045,662	1.43 (1.40-1.45)
Mexican	10,357	643,677	1.27 (1.23-1.31)	22,336	1,521,220	1.39 (1.37-1.42)
Other Hispanic	5,425	277,346	1.38 (1.33-1.44)	9,829	524,442	1.51 (1.48-1.55)
Foreign-born				29,960	2,382,769	1.23 (1.20-1.25)
Mexican				22,395	1,955,264	1.15 (1.13-1.17)
Other Hispanic				7,565	427,505	1.52 (1.48-1.56)
White						
US-born	6,936	496,444	Reference	26,760	2,204,279	Reference
Foreign-born				4,192	330,124	0.96 (0.93-1.00)

Note: Mexican and other Hispanic are subsets of grandmothers who were Hispanic and Filipino is a subset of grandmothers who were Asian Pacific Islander. OR = odds ratio.

[a] Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female participants), mother's parity (1, 2, 3 or more), and mother's age (18-24, 25-29, 30-34, 35-55 years).

Figure 2-S1. California Department of Developmental Services Autism Spectrum Disorders to California Birth Certificate Linkage

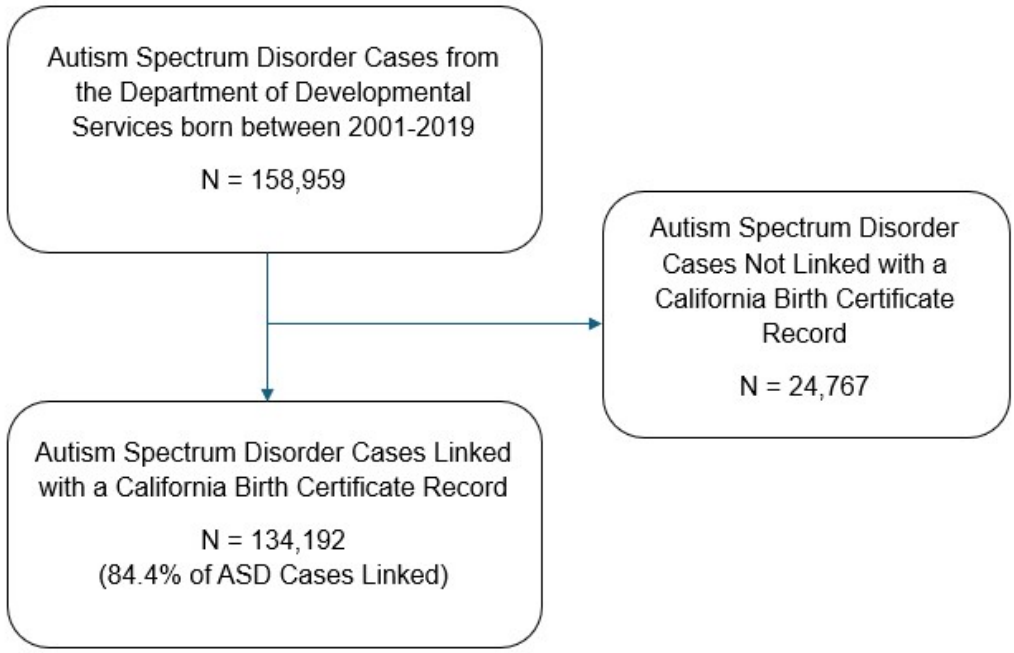


Figure 2-S2. Multigenerational Birth Linkage

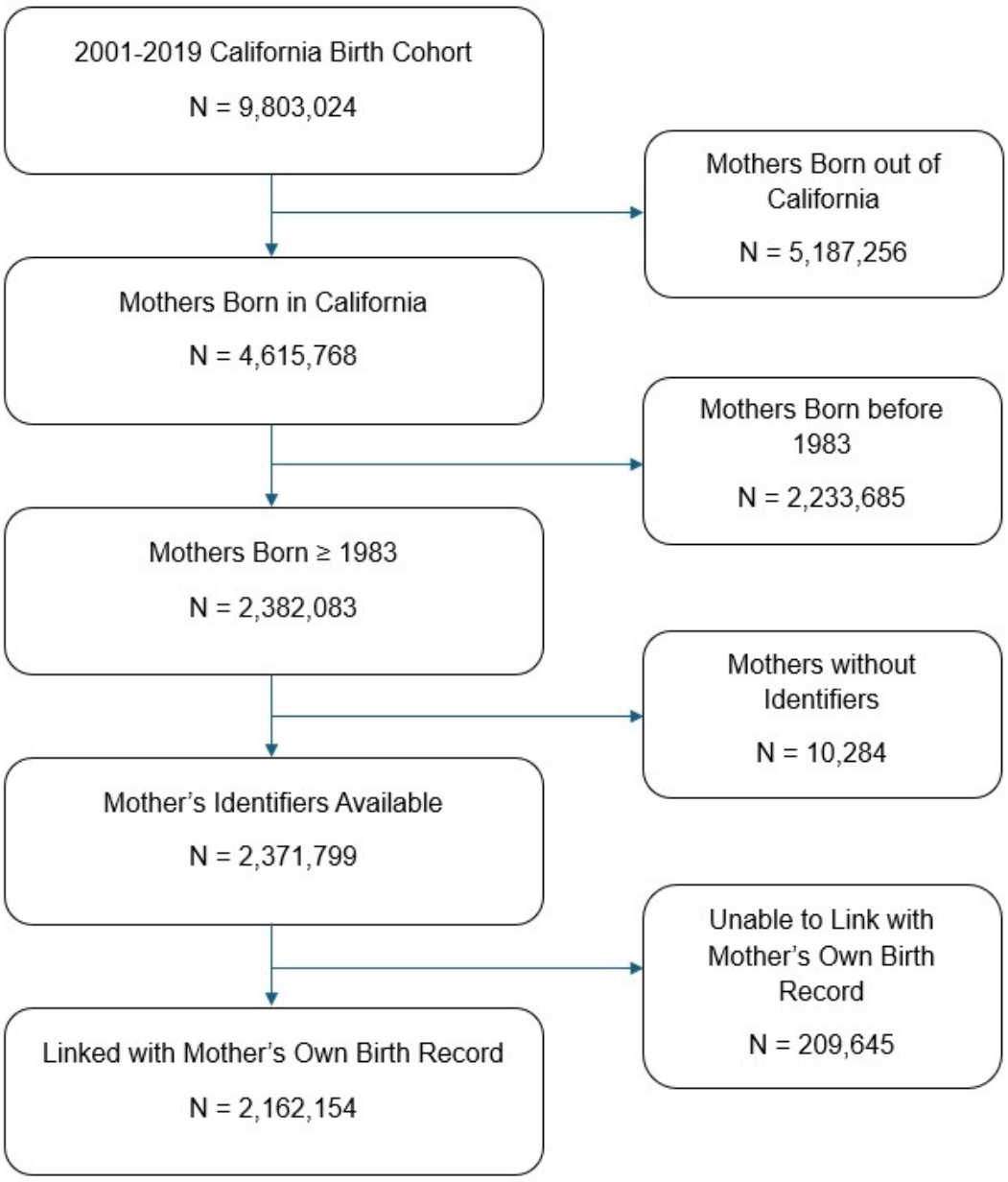
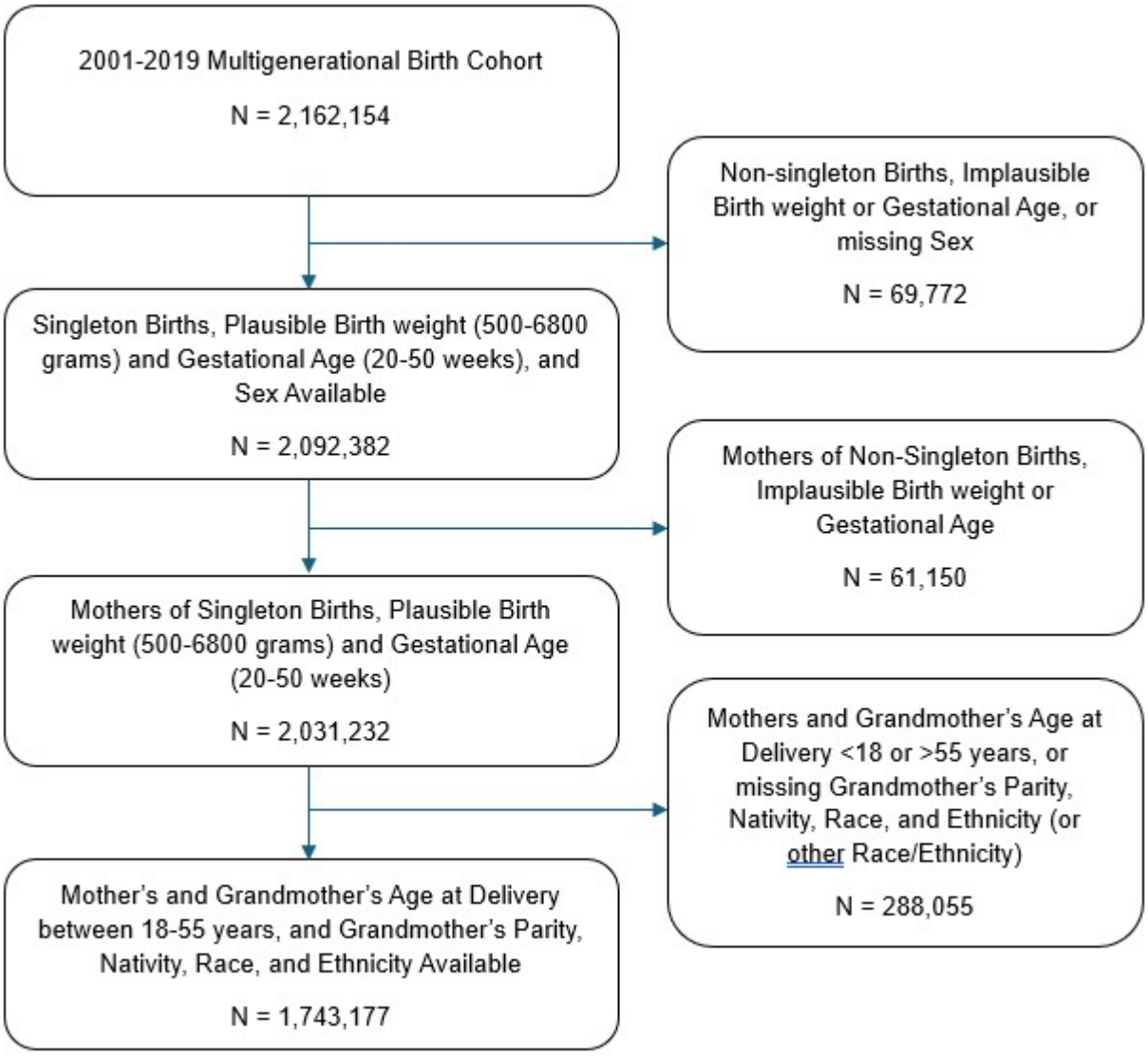


Figure 2-S3. Study Population Flowchart



3. Autism Spectrum Disorders and Grandparents' Age, Race, and Ethnicity

3.1. Introduction

Autism spectrum disorders (ASD) is a neurodevelopmental disorder characterized by repetitive behaviors and restricted interests, and difficulties in social communication and interaction [2,3]. The risk of ASD has been steadily increasing over time [16] with an estimated prevalence of 27.6 per 1000 children in the United States and 44.9 per 1000 in California in 2020 (among children born in 2012) [15]. Parental age at childbirth has been increasing in past decades as well [72-74], and multiple studies have reported associations between older parental age and ASD risk in offspring [75,76].

Interestingly, there is growing evidence that the age at which grandparents conceived the maternal generation may also be associated with ASD risk in grandchildren [33-35]. Potential mechanisms for multigenerational disease transmission include germ cell reprogramming through the parental germline [42], cumulative mitochondrial DNA (mtDNA) damage during in-utero oocyte development for matrilineal transmission [44], or de novo mutations or epigenetic programming of the sperm transmitted patrilineally [45]. Through these proposed mechanisms, environmental exposures may eventually exhibit adverse effects on brain development in offspring and may accumulate with age [77] to exhibit multigenerational influences [46]. Environmental exposures are commonly concentrated where minority populations live in California [24].

Previously, studies found higher risks of ASD in grandchildren of younger and older maternal grandparents but these studies were limited to European-ancestry and predominately White populations [33-35]. Here, we evaluate the risk of ASD in grandchildren with maternal grandparents' age at the time when their daughter was born with a focus on identifying differences by race/ethnicity. Our ethnically and racially diverse California study population will

allow us to better understand whether these age-effects apply universally and/or are proxies for biologic or environmental risk factors that accumulate with age. We hypothesize that the risk of ASD among grandchildren will be higher among younger (<25 years) and older (≥35 years) grandparents compared to grandparents ages 25-29 years, and that this pattern will differ by race/ethnicity.

3.2. Methods

Study Population

This is a multigenerational California birth cohort study of children born from 2001-2019 by mothers who were born from 1983-2001. We linked 2002-2022 ASD diagnostic records from the California Department of Developmental Services (DDS) [7] to 2001-2019 California birth records from the California Department of Public Health [53] using similar probabilistic linkage methods (with National Program of Cancer Registries Registry Plus Link Plus Software [55]) as described by other studies [56] based on child and parental identifiers (Table 2-S1). Birth records that did not link with a DDS ASD diagnostic record were considered non-cases (Figure 2-S1). ASD cases include individuals with a primary diagnosis of autistic disorder under the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised (DSM-IV-R) until 2014 or the DSM-V [2,5]. We then linked each birth to the mother's own California birth record using maternal information found on the birth record (Figure 2-S2).

All California born offspring and mothers were eligible for this study if they had been singleton births with plausible birthweight (500-6800 grams) and gestational age (20-50 weeks), mother's and grandmother's ages were 18-55 years, and information on child's sex, grandmother's parity, race, and ethnicity were available. We further required that the grandfather's age at mother's delivery was between 18-55 years and information on grandfather's race and ethnicity was available for the analysis of grandfather's age. Our final analytical study population included

1,743,998 mother-child pairs with complete information for analysis of grandmother's age and 1,630,722 mother-child pairs with complete information for analysis of grandfather's age (Figure 3-S1). Maternal grandmother's and grandfather's ages were recorded on the mother's birth record.

Statistical Analysis

We performed logistic regression analyses to obtain odds ratios (ORs) and 95% CIs for ASD adjusting for child's birth year and sex (male, female), mother's birth year, grandmother's parity (1,2,3 or more), and grandmother's or grandfather's respective race/ethnicity (Asian Pacific Islander (API), Black, Hispanic, White). Hispanic grandparents are Hispanic of any race. Covariates were recorded on the grandchild's or mother's birth records and included in the regression models as categorical (see above) or continuous (birth year). Grandmother's or grandfather's ages were categorized as 18-24 (younger), 25-29, 30-34, and 35-55 (older) years, with ages 25-29 years as the reference group since grandmothers were on average 26 years and grandfathers 29 years of age when their daughter was born (Table 3-S1). We evaluated plots of the crude percent of ASD for each grandparents' age at 1-year intervals and found that the risk of ASD in grandchildren by maternal grandparents' age was non-linear (Figure 3-S2). We additionally performed logistic regression using cubic restricted splines with varying knots spaced at equal percentiles to see if the model fit would be improved. Model fit was evaluated by the Akaike information criterion (AIC), where a lower AIC indicates a better model fit.

For sensitivity analyses, we mutually adjusted for the other grandparent's age and for the mother's age at birth of the grandchild to assess whether the associations for grandmother's or grandfather's age were independent of each other or of the mother's age. We also adjusted for grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more) among those for whom data was available i.e.

beginning with 1989 births. We also conducted sensitivity analyses by including grandfathers ages >55 years since some men fathered children much later in life. To account for outcome correlatedness, we restricted some analyses to firstborn grandchildren.

We investigated effect measure modification of grandmother's or grandfather's age and grandchild's ASD by grandparent's race/ethnicity by adding interaction terms into the regression model. Grandparents aged 25-29 years and of White race/ethnicity were used as the reference group for all analyses because current multigenerational studies have been conducted predominantly in White populations. An interaction term with a 2-sided Wald chi-square p-value <0.10 for heterogeneity was considered statistically significant for the overall joint test and for each age and race/ethnicity interaction term. Finally, we repeated the main analysis after stratifying by grandparent's race/ethnicity and plotted the estimated ORs and 95% CIs on the log scale.

Data analysis was performed using SAS 9.4 (SAS Institute Inc.). This study has been approved by the California State and University of California Los Angeles Institutional Review Boards with waiver of consent.

3.3. Results

Among our study population of 1,743,998 children in the analysis for grandmother's age, 852,958 were female (48.9%) and 27,975 children were identified as ASD cases (1.6%) (Table 3-1). Among cases, 6,016 were female (21.5%). Mothers of all children were born between 1983-2001 with 554,272 being the firstborn child of the grandmother (31.8%) (Table 3-1, Table 3-S2). The majority of grandmothers were of Hispanic ethnicity of any race followed by grandmothers of White race. The demographic distributions were similar for the smaller subset of 1,630,722 children included in the analysis for grandfather's age (Table 3-1).

The odds of ASD in grandchildren were slightly elevated among grandmothers who gave birth to the mothers when they were younger (OR, 1.03; 95% CI, 1.00-1.06) or older (OR, 1.09; 95% CI, 1.04-1.15) compared to grandmothers 25-29 years of age at the mother's delivery, adjusting for child's birth year and sex, mother's birth year, grandmother's parity, and grandmother's race/ethnicity (Table 3-2). Results remained similar with or without adjusting for mother's age and grandmother's education (Table 3-S3, Table 3-S4). Results after adjusting for grandfather's age also remained similar, except the effect estimate for older grandmothers became slightly attenuated (Table 3-S5). Results among firstborn grandchildren also remained similar (Table 3-S6). When we performed logistic regression using restricted cubic splines with 4, 5, and 6 evenly spaced knots, we found that the AIC remained similar (Table 3-S7).

The overall joint interaction term for grandmother's age and race/ethnicity was statistically significant ($p<0.0001$) as were the following race/ethnicity and age group interaction terms: API grandmothers 18-24 years ($p=0.04$), Black grandmothers 18-24 years ($p<0.0001$), and Hispanic grandmothers 18-24 years ($p<0.0001$) (Table 3-3). In subgroup analyses stratified by race/ethnicity, many of the 95% CIs crossed the null. However, we observed elevated risks of ASD in grandchildren among younger and older White, older API, and older Hispanic grandmothers but lower risks among younger and no differences in older Black grandmothers (Figure 3-1, Table 3-S3).

The odds of ASD in grandchildren were also higher among younger grandfathers (OR, 1.04; 95% CI, 1.01-1.08) and older grandfathers (OR, 1.08; 95% CI, 1.04-1.12) compared to grandfathers ages 25-29 years at the mother's delivery, adjusting for child's birth year and sex, mother's birth year, grandmother's parity, and grandfather's race/ethnicity (Table 3-2). Results remained similar with or without adjustment for the mother's age, grandmother's age, and

grandmother's education (Table 3-S8, Table 3-S9). Results for older grandfathers without restricting to ≤55 years of age and results among firstborn grandchildren also remained similar (Table 3-S10, Table 3-S11). Logistic regressions using restricted cubic splines with 4, 5, and 6 evenly spaced knots instead of age categories had a similar fit according to the AIC (Table 3-S7).

The interaction term for grandfather's age and race/ethnicity was statistically significant for the overall joint test ($p<0.0001$) and for the following race/ethnicity and age groups: API grandfathers 18-24 years ($p=0.001$), Black grandfathers 18-24 years ($p<0.0001$), Hispanic grandfathers 18-24 years ($p<0.0001$), Black grandfathers 35-55 years ($p=0.09$), and Hispanic grandfathers 35-55 years ($p=0.05$) (Table 3-3). We observed higher risks of ASD in grandchildren among younger White, older Black, and older Hispanic grandfathers (Figure 3-2, Table 3-S8).

3.4. Discussion

We found that in California, the risk of ASD in grandchildren was elevated among younger (18-24 years) and older (35-55 years) maternal grandparents compared to grandparents 25-29 years of age corroborating previous reports from studies of mainly White European ancestry populations, but our results also varied across racial and ethnic groups. Among White grandparents, the risk of ASD in grandchildren was higher at both ends of the age spectrum while for Hispanic grandparents and API grandmothers the risk of ASD in grandchildren was higher only among older grandparents. Interestingly, among Black grandparents, the risk of ASD in grandchildren was lowest for the youngest grandmothers and higher only for older grandfathers.

The increased overall ASD risk we observed in grandchildren of younger grandparents confirm results previously reported in a Danish study population [34]. Interestingly, this increase for younger grandparents was driven by White grandparents while we did not observe this for any of the minority grandparents, with inverse associations for younger Black grandmothers and no observed differences for younger Hispanic grandparents compared the referent age group. Among White grandparents, younger age at birth may reflect lower socioeconomic status (SES) since White women of advantageous socioeconomic background are more likely to delay childbirth [78] while early motherhood has been associated with unfavorable socioeconomic conditions and poorer life outcomes [79,80]. In support of this theory, our effect estimates for younger (but not older) White grandparents were slightly attenuated after adjusting for grandmother's education.

Our results confirm that maternal grandmother's older age at birth may influence ASD risk in grandchildren as previously reported in a United Kingdom study population [35]. Results from a Danish study found that the risk of ASD in grandchildren was elevated for maternal grandmother's ≥40 years of age [34]. Our estimated effect size was weaker for older White grandmothers and the confidence interval included the null, but we also observed the same age effect in Hispanic grandmothers in California. Our results also corroborate previous results in a Swedish study population [33] that older maternal grandfather's age may influence ASD risk in grandchildren, but again our results had a smaller effect size and the confidence interval included the null similar to what was seen in the Danish population [34].

Adverse associations were seen for all older grandparents except for older Black grandmothers and API grandfathers. Elevated risks of ASD among grandchildren of older maternal grandparents have been suggested to be due to germ cell reprogramming (epigenetic marks) [42], impaired mitochondrial function (for grandmothers) [44], with consequences for oocyte and

sperm quality [26,27]. For example, mitochondrial DNA mutations during in-utero oocyte development may accumulate with age and be passed on matrilineally [43,44].

The stronger positive associations seen for older Hispanic grandparents and Black grandfathers but not for White or API grandparents perhaps additionally suggest environmental and occupational exposures in these generally lower SES groups that may elevate risks among older grandparents. Environment may have a multigenerational influence on health outcomes potentially through altered epigenetic regulation of the germline [46]. Studies have reported higher environmental exposures among minority populations in California [24] which may explain differences in effect size by race/ethnicity, though it is unclear if germ cell reprograming may differ by race/ethnicity. While we do not have occupational data, it is plausible that occupational factors may play a role. According to the 1987 and 1980 Current Population Survey (CPS) by the Bureau of Labor Statistics, higher proportions of Hispanic men and women (compared to non-Hispanic men and women) and Black men (compared to White men) were working in blue collar jobs or service work [81,82]. There was an increase in the incarceration rate in the 1980s with a disproportionate incarceration of Black and other minority Americans [83], and racism and discrimination can lead to higher levels of distress and adverse mental health outcomes with adverse behavioral and lifestyle choice consequences [84]. In murine models, conditioned fear or stress during in-utero development of the parental generation has been reported to lead to altered behavior in the grandchildren generation [38].

Our results for maternal grandparents' age and ASD in grandchildren hold even after adjusting for mother's age, while effect estimates for older grandmothers weakened after adjusting for grandfather's age perhaps suggesting that part of the maternal grandmother's older age effect is explained by older grandfather's age. In contrast, the effect estimates for older maternal grandfathers generally did not get weaker after adjustment for grandmother's age (except for

Hispanic grandfathers). In our study population, mother's age at her child's delivery is not strongly correlated with grandmother's (r=0.096) or grandfather's (r=0.092) ages at mother's delivery; however, grandmother's and grandfather's ages are correlated (r=0.645) (Table 3-S12). The reduction in the estimated grandmaternal age effect after adjustment for grandfather's age may indicate that the biological mechanism for older grandmother's age is explained in part by a contribution from grandfather's age at least in the maternal line. While environmental influences may interfere with epigenetic reprogramming that occurs after fertilization [39], altered germline epigenetics may be more applicable for aging grandfathers than aging grandmothers since sperm are generated throughout adolescents to adulthood while oocyte development is set in-utero development [85]. However, more research is needed to tease apart the different mechanisms of the maternal and paternal lines or how these mechanisms interact with each other.

Our results did not change more than minimally in multiple sensitivity analyses. However, there may still be uncontrolled confounding due to risk factors not recorded on the birth certificates. ASD identification by the DDS has a high specificity (low false positive rate) since assessments at the DDS are made by well-trained interdisciplinary diagnostic teams and only qualified professions can make an autism diagnosis in California [11]. However, sensitivity of case identification may have changed over the years with growing ASD awareness in the population in general. As the prevalence of ASD has been increasing over the last decades while the age at first ASD diagnosis has been decreasing [17], we adjusted for child's birth year to address this strong risk factor. We did not adjust age at ASD diagnosis in grandchildren as it cannot affect grandparent's age and therefore is not a confounder.

Our California multigenerational birth cohort can uniquely investigate associations by race/ethnicity due to the racially and ethnically diverse population of California. Differences by

race/ethnicity may imply different mechanisms underlying the association between grandparental age and ASD in grandchildren. Additional strengths of this study include its California wide coverage and relatively large sample size. Maternal grandparents' age and other covariates from the grandparents' generation were obtained from birth records which eliminates the possibility of recall bias. Using California birth records also reduced the possibility of participation bias since women were not self-selecting to participate in our study and cases and non-cases were enrolled according to the same eligibility criteria.

Despite our overall large sample size, our Black and API population analyses may have lacked statistical power. Yet, we did observe lower risk among younger Black grandmothers and higher risk among older Black grandfathers. Additional limitations include the truncation of maternal age (and potentially of grandparental age) due to electronic data availability starting with 1983 births (Table 3-S13). Since older maternal age is associated with offspring ASD, this may lead to a higher proportion of older mothers with ASD offspring to not be included in our multigenerational study population. If mother's and grandparents' ages at birth are correlated, a greater number of older grandparents with a grandchild with ASD might have not been included possibly resulting in attenuation of our effect estimates for older grandparents.

We also had to restrict our birth cohort to mothers born in California (Figure 2-S2) to link birth records of mothers and their children. A higher proportion of mothers born in California were White and more educated (high school or more) compared to mothers not born in California (Table 3-S14). This may have resulted in a greater proportion of older grandparents in our multigenerational study population. We also focused on the maternal and not the paternal line to identify grandparents since paternal identifiers became available and/or are more complete in later birth years, leaving these investigations to future studies.

3.5. Conclusion

Our study confirms that older maternal grandparents' age at the time of the mother's birth is positively associated with ASD risk in the grandchildren. We also saw increases in ASD risk for children whose mothers were born to younger White maternal grandparents as previously reported. In addition, patterns differed according to race/ethnicity indicating that the risk of ASD in grandchildren observed for younger and older age grandparents may reflect different multigenerational mechanisms. However, future studies with biological, social, environmental, and occupational data are needed to elucidate these mechanisms.

3.6. Tables and Figures

Table 3-1. Study Population Characteristics by Autism Spectrum Disorders

	Analysis for Grandmother's Age		Analysis for Grandfather's Age	
Study Population Characteristics	**Case (N=27975)**	**Total (N=1743998)**	**Case (N=25816)**	**Total (N=1630722)**
Child's Sex, n (%)				
Female	6016 (21.5%)	852958 (48.9%)	5544 (21.5%)	797754 (48.9%)
Male	21959 (78.5%)	891040 (51.1%)	20272 (78.5%)	832968 (51.1%)
Mother's Birth Year, mean (SD)	1988.6 (4.00)	1988.2 (3.94)	1988.5 (3.98)	1988.1 (3.91)
Grandmother's Parity, n (%)				
1	9092 (32.5%)	554272 (31.8%)	8235 (31.9%)	508526 (31.2%)
2	8431 (30.1%)	539547 (30.9%)	7871 (30.5%)	511425 (31.4%)
3 or more	10452 (37.4%)	650179 (37.3%)	9710 (37.6%)	610771 (37.5%)
Grandmother's Race/Ethnicity, n (%)				
Asian Pacific Islander	1302 (4.7%)	92593 (5.3%)	1207 (4.7%)	86045 (5.3%)
Black	2616 (9.4%)	151690 (8.7%)	2380 (9.2%)	139195 (8.5%)
Hispanic of any race	15217 (54.4%)	887421 (50.9%)	13940 (54.0%)	823195 (50.5%)
White	8840 (31.6%)	612294 (35.1%)	8289 (32.1%)	582287 (35.7%)
Grandfather's Race/Ethnicity, n (%)				
Asian Pacific Islander	1207 (4.4%)	85473 (5.0%)	1164 (4.5%)	82565 (5.1%)
Black	2809 (10.3%)	165902 (9.8%)	2654 (10.3%)	156792 (9.6%)
Hispanic of any race	15012 (55.3%)	877401 (51.6%)	14198 (55.0%)	836424 (51.3%)
White	7965 (29.3%)	564037 (33.2%)	7800 (30.2%)	554941 (34.0%)
Other	162 (0.6%)	8426 (0.5%)		
Missing	820	42759		

Table 3-2. Maternal Grandparents' Age and Autism Spectrum Disorders in Grandchildren

	Children, n		**Odds Ratio (95% CI)**		
Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 2**
18-24 years	12654	783693	1.06 (1.03-1.09)	1.06 (1.03-1.09)	1.03 (1.00-1.06)
25-29 years	8384	535350	Reference	Reference	Reference
30-34 years	4735	298166	0.99 (0.96-1.03)	0.99 (0.96-1.03)	1.00 (0.97-1.04)
35-55 years	2202	126789	1.09 (1.04-1.14)	1.08 (1.03-1.14)	1.09 (1.04-1.15)
Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 2**
18-24 years	7539	466935	1.07 (1.04-1.11)	1.07 (1.04-1.11)	1.04 (1.01-1.08)
25-29 years	8212	533729	Reference	Reference	Reference
30-34 years	5685	366087	0.99 (0.96-1.03)	0.99 (0.96-1.03)	1.01 (0.98-1.05)
35-55 years	4380	263971	1.06 (1.02-1.10)	1.05 (1.01-1.09)	1.08 (1.04-1.12)

Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandparent's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

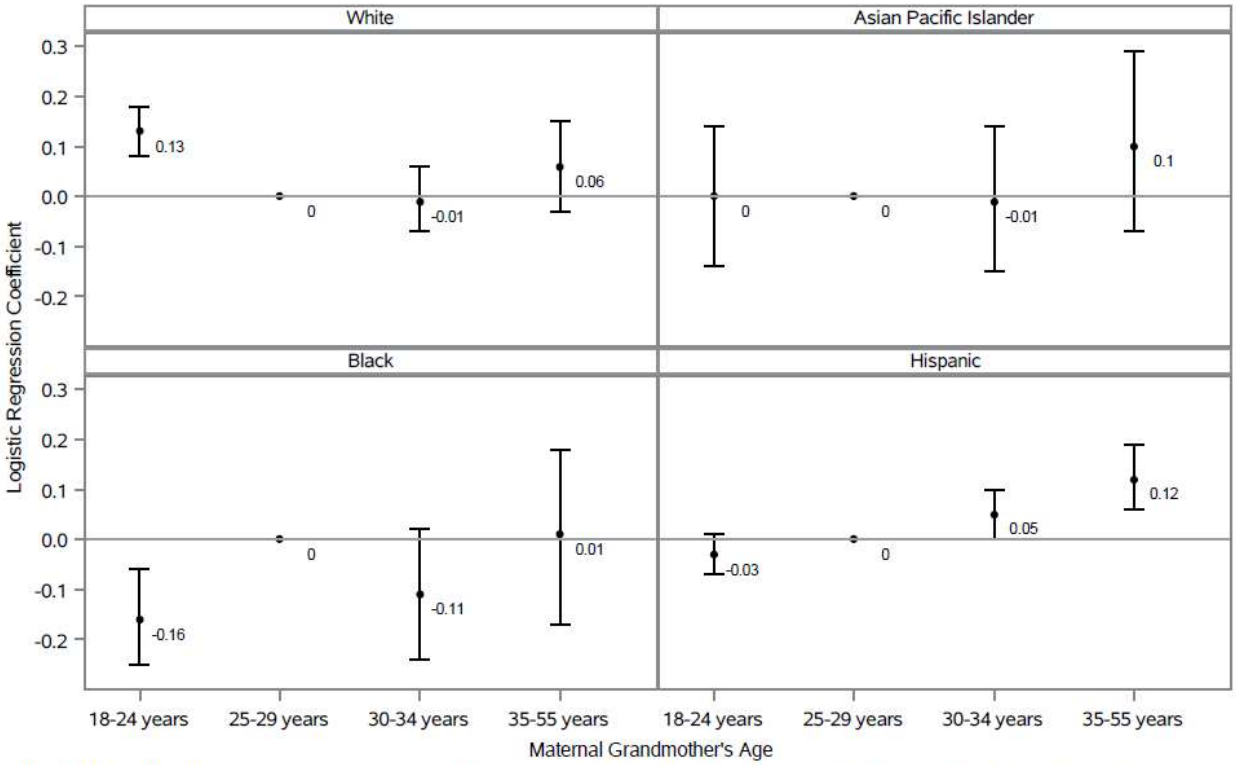
Table 3-3. Interaction P-values for Maternal Grandparents' Age and Race/Ethnicity with Autism Spectrum Disorders in Grandchildren

Grandmother's Age and Race/Ethnicity							
		Children, n					
Interaction Terms		**Cases**	**Total**	**Coefficient**	**Standard Error**	**P-value**	
18-24 years	Asian Pacific Islander	389	28233	-0.16	0.08	0.04	*
18-24 years	Black	1309	80165	-0.32	0.05	<0.0001	*
18-24 years	Hispanic of any race	7184	431210	-0.20	0.03	<0.0001	*
30-34 years	Asian Pacific Islander	313	22521	0.00	0.08	0.96	
30-34 years	Black	362	21243	-0.10	0.07	0.15	
30-34 years	Hispanic of any race	2453	136954	0.06	0.04	0.12	
35-55 years	Asian Pacific Islander	189	12399	0.04	0.10	0.69	
35-55 years	Black	156	8368	-0.10	0.10	0.32	
35-55 years	Hispanic of any race	1248	65013	0.05	0.06	0.41	
Overall Joint Test						<0.0001	*
Grandfather's Age and Race/Ethnicity							
		Children, n					
Interaction Terms		**Cases**	**Total**	**Coefficient**	**Standard Error**	**P-value**	
18-24 years	Asian Pacific Islander	171	13054	-0.32	0.10	0.001	*
18-24 years	Black	886	56132	-0.29	0.06	<0.0001	*
18-24 years	Hispanic of any race	4539	274576	-0.21	0.04	<0.0001	*
30-34 years	Asian Pacific Islander	344	24463	-0.09	0.08	0.30	
30-34 years	Black	486	29194	-0.02	0.07	0.72	
30-34 years	Hispanic of any race	2898	168204	0.04	0.04	0.27	
35-55 years	Asian Pacific Islander	298	21744	-0.13	0.09	0.14	
35-55 years	Black	492	25056	0.11	0.07	0.09	*
35-55 years	Hispanic of any race	2224	120056	0.09	0.04	0.05	*
Overall Joint Test						<0.0001	*

*P-values that are statistically significant (<0.10).

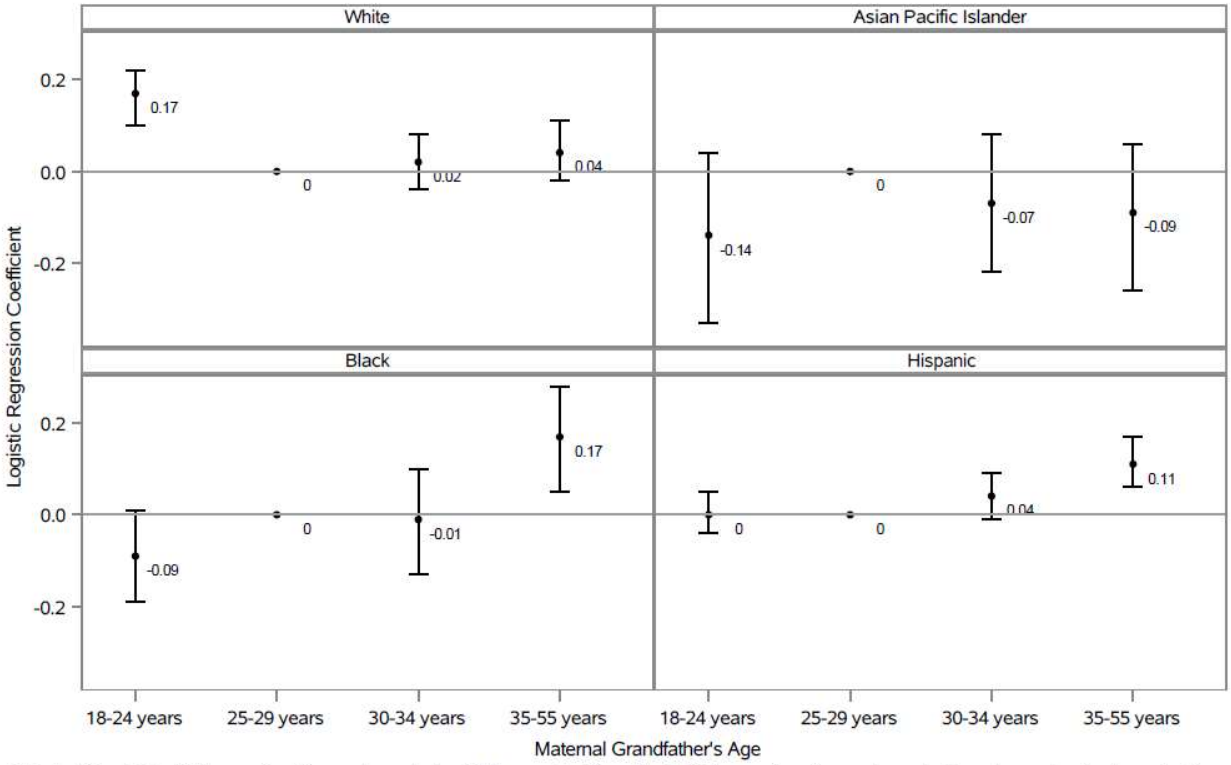
Note: All models are adjusted for offspring birth year (continuously scaled), offspring sex (male, female), mother's birth year (continuously scaled), and grandmother's parity (1, 2, 3 or more). Grandmothers or grandfathers 25-29 years and of White race/ethnicity were the reference group, with a sample size of 244,085 (3,772 cases) and 123,173 (1,943) respectively.

Figure 3-1. Maternal Grandmother's Age and Autism Spectrum Disorders in Grandchildren



Adjusted for child's birth year (continuously scaled), child's sex (M, F), mother's birth year (continuously scaled), and grandmother's parity (1, 2, 3 or more)

Figure 3-2. Maternal Grandfather's Age and Autism Spectrum Disorders in Grandchildren



Adjusted for child's birth year (continuously scaled), child's sex (M, F), mother's birth year (continuously scaled), and grandmother's parity (1, 2, 3 or more)

3.7. Supplemental Materials

Table 3-S1. Maternal Grandmother and Grandfather's Age by Race/Ethnicity

	Asian Pacific Islander	**Black**	**Hispanic of any race**	**White**	**Total**
Grandmother's Age (in years)	(N=92593)	(N=151690)	(N=887421)	(N=612294)	(N=1743998)
Continuous, mean (SD)	27.9 (5.72)	24.9 (5.19)	25.6 (5.39)	26.3 (5.04)	25.9 (5.31)
Categorical, n (%)					
18-24 years	28233 (30.5%)	80165 (52.8%)	431210 (48.6%)	244085 (39.9%)	783693 (44.9%)
25-29 years	29440 (31.8%)	41914 (27.6%)	254244 (28.6%)	209752 (34.3%)	535350 (30.7%)
30-34 years	22521 (24.3%)	21243 (14.0%)	136954 (15.4%)	117448 (19.2%)	298166 (17.1%)
35-55 years	12399 (13.4%)	8368 (5.5%)	65013 (7.3%)	41009 (6.7%)	126789 (7.3%)
Grandfather's Age (in years)	(N=82565)	(N=156792)	(N=836424)	(N=554941)	(N=1630722)
Continuous, mean (SD)	31.0 (6.45)	28.0 (6.83)	28.0 (6.13)	29.2 (5.87)	28.5 (6.18)
Categorical, n (%)					
18-24 years	13054 (15.8%)	56132 (35.8%)	274576 (32.8%)	123173 (22.2%)	466935 (28.6%)
25-29 years	23304 (28.2%)	46410 (29.6%)	273588 (32.7%)	190427 (34.3%)	533729 (32.7%)
30-34 years	24463 (29.6%)	29194 (18.6%)	168204 (20.1%)	144226 (26.0%)	366087 (22.4%)
35-55 years	21744 (26.3%)	25056 (16.0%)	120056 (14.4%)	97115 (17.5%)	263971 (16.2%)

Table 3-S2. Offspring and Mother's Birth Year

Child's Birth Year, n (%)	**Total (N=1743998)**
2001	2875 (0.2%)
2002	10024 (0.6%)
2003	18871 (1.1%)
2004	28257 (1.6%)
2005	38281 (2.2%)
2006	50834 (2.9%)
2007	60493 (3.5%)
2008	74950 (4.3%)
2009	84502 (4.8%)
2010	94318 (5.4%)
2011	104422 (6.0%)
2012	116263 (6.7%)
2013	126568 (7.3%)
2014	138590 (7.9%)
2015	147043 (8.4%)
2016	155810 (8.9%)
2017	160517 (9.2%)
2018	162877 (9.3%)
2019	168503 (9.7%)
Mother's Birth Year, n (%)	**Total (N=1743998)**
1983	183302 (10.5%)
1984	176646 (10.1%)
1985	171971 (9.9%)
1986	160963 (9.2%)
1987	151386 (8.7%)
1988	144920 (8.3%)
1989	138512 (7.9%)
1990	131045 (7.5%)

1991	113067 (6.5%)
1992	94407 (5.4%)
1993	77592 (4.4%)
1994	62139 (3.6%)
1995	47486 (2.7%)
1996	35468 (2.0%)
1997	25122 (1.4%)
1998	15580 (0.9%)
1999	9133 (0.5%)
2000	4205 (0.2%)
2001	1054 (0.1%)

Table 3-S3. Maternal Grandmother's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity

	Children, n		Odds Ratio (95% CI)		
Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 2**	**Model 3A**
18-24 years	12654	783693	1.06 (1.03-1.09)	1.03 (1.00-1.06)	1.03 (1.00-1.06)
25-29 years	8384	535350	Reference	Reference	Reference
30-34 years	4735	298166	0.99 (0.96-1.03)	1.00 (0.97-1.04)	1.00 (0.97-1.04)
35-55 years	2202	126789	1.09 (1.04-1.14)	1.09 (1.04-1.15)	1.10 (1.04-1.15)
White Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**
18-24 years	3772	244085	1.15 (1.10-1.21)	1.14 (1.08-1.20)	1.14 (1.08-1.20)
25-29 years	2852	209752	Reference	Reference	Reference
30-34 years	1607	117448	1.00 (0.94-1.06)	0.99 (0.93-1.06)	1.00 (0.94-1.06)
35-55 years	609	41009	1.09 (1.00-1.19)	1.06 (0.97-1.16)	1.07 (0.98-1.17)
Asian Pacific Islander Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**
18-24 years	389	28233	1.01 (0.88-1.16)	1.00 (0.87-1.15)	0.99 (0.86-1.15)
25-29 years	411	29440	Reference	Reference	Reference
30-34 years	313	22521	0.98 (0.85-1.14)	0.99 (0.86-1.15)	1.00 (0.86-1.16)
35-55 years	189	12399	1.10 (0.92-1.31)	1.11 (0.93-1.33)	1.11 (0.93-1.33)
Black Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**
18-24 years	1309	80165	0.88 (0.80-0.96)	0.85 (0.78-0.94)	0.86 (0.78-0.94)
25-29 years	789	41914	Reference	Reference	Reference
30-34 years	362	21243	0.87 (0.77-0.99)	0.90 (0.79-1.02)	0.90 (0.79-1.02)
35-55 years	156	8368	0.94 (0.79-1.12)	1.01 (0.84-1.20)	1.01 (0.84-1.20)
Hispanic Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**
18-24 years	7184	431210	0.99 (0.95-1.03)	0.97 (0.93-1.01)	0.97 (0.93-1.01)
25-29 years	4332	254244	Reference	Reference	Reference
30-34 years	2453	136954	1.04 (0.99-1.09)	1.05 (1.00-1.11)	1.05 (1.00-1.11)
35-55 years	1248	65013	1.11 (1.04-1.18)	1.13 (1.06-1.21)	1.13 (1.06-1.21)

Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Model 3A adjusts for the variables in model 2 plus mother's age (18-24, 25-29, 30-34, 35-55 years).

Model 3B adjusts for the variables in model 1 plus mother's age (18-24, 25-29, 30-34, 35-55 years).

Table 3-S4. Maternal Grandmother's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity, with Grandmother's Education Information

	Children, n		Odds Ratio (95% CI)	
Grandmother's Age	**Cases**	**Total**	**Model 2**	**Model 3A**
18-24 years	6144	348815	1.00 (0.96-1.05)	0.98 (0.94-1.02)
25-29 years	3766	216516	Reference	Reference
30-34 years	2209	124299	1.03 (0.97-1.08)	1.04 (0.99-1.10)
35-55 years	1098	58384	1.09 (1.02-1.17)	1.12 (1.04-1.20)
White Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	1463	83279	1.08 (0.99-1.17)	1.02 (0.94-1.11)
25-29 years	1031	63310	Reference	Reference
30-34 years	594	38365	0.95 (0.86-1.05)	0.97 (0.88-1.08)
35-55 years	250	15311	0.99 (0.86-1.14)	1.04 (0.91-1.20)
Asian Pacific Islander Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	160	10626	1.14 (0.88-1.47)	1.13 (0.87-1.46)
25-29 years	105	7831	Reference	Reference
30-34 years	84	5846	1.07 (0.80-1.44)	1.09 (0.81-1.46)
35-55 years	57	4019	1.08 (0.78-1.50)	1.08 (0.77-1.50)
Black Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	579	34261	0.84 (0.73-0.96)	0.83 (0.72-0.96)
25-29 years	351	17702	Reference	Reference
30-34 years	175	10448	0.84 (0.70-1.01)	0.84 (0.70-1.01)
35-55 years	92	4565	1.03 (0.81-1.30)	1.02 (0.81-1.30)
Hispanic Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	3942	220649	0.98 (0.93-1.04)	0.97 (0.92-1.03)
25-29 years	2279	127673	Reference	Reference
30-34 years	1356	69640	1.10 (1.03-1.18)	1.11 (1.03-1.19)
35-55 years	699	34489	1.15 (1.06-1.26)	1.17 (1.07-1.28)

Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female), mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Model 3A adjusts for the variables in model 2 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more).

Model 3B adjusts for the variables in model 1 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more).

Table 3-S5. Maternal Grandmother's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity, with Grandfather's Age Information

	Children, n		Odds Ratio (95% CI)	
Grandmother's Age	**Cases**	**Total**	**Model 2**	**Model 3A**
18-24 years	11479	719248	1.03 (1.00-1.06)	1.03 (0.99-1.06)
25-29 years	7903	509815	Reference	Reference
30-34 years	4430	283718	0.99 (0.96-1.03)	0.98 (0.94-1.02)
35-55 years	2004	117941	1.08 (1.03-1.13)	1.04 (0.98-1.10)
White Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	3442	226480	1.13 (1.07-1.19)	1.08 (1.02-1.15)
25-29 years	2734	202797	Reference	Reference
30-34 years	1534	113857	0.99 (0.93-1.05)	0.98 (0.91-1.05)
35-55 years	579	39153	1.07 (0.97-1.17)	1.05 (0.95-1.17)
Asian Pacific Islander Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	346	24881	1.01 (0.87-1.17)	1.02 (0.86-1.20)
25-29 years	390	28100	Reference	Reference
30-34 years	301	21688	1.00 (0.86-1.17)	1.02 (0.86-1.20)
35-55 years	170	11376	1.10 (0.91-1.32)	1.15 (0.93-1.43)
Black Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	1198	73460	0.86 (0.78-0.94)	0.89 (0.80-1.00)
25-29 years	727	38936	Reference	Reference
30-34 years	317	19295	0.87 (0.76-1.00)	0.83 (0.72-0.95)
35-55 years	138	7504	1.01 (0.84-1.21)	0.89 (0.73-1.08)
Hispanic Grandmother's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	6493	394427	0.97 (0.93-1.01)	0.98 (0.94-1.03)
25-29 years	4052	239982	Reference	Reference
30-34 years	2278	128878	1.04 (0.99-1.10)	1.02 (0.96-1.08)
35-55 years	1117	59908	1.11 (1.03-1.18)	1.05 (0.98-1.13)

Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female), mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Model 3A adjusts for the variables in model 2 plus grandfather's age (18-24, 25-29, 30-34, 35-55 years).

Model 3B adjusts for the variables in model 1 plus grandfather's age (18-24, 25-29, 30-34, 35-55 years).

Table 3-S6. Maternal Grandmother's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity, among Firstborn Grandchildren

	Children, n		Odds Ratio (95% CI)	
Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 2**
18-24 years	6631	377340	1.07 (1.03-1.11)	1.06 (1.02-1.10)
25-29 years	4704	276282	Reference	Reference
30-34 years	2757	160756	0.98 (0.93-1.03)	0.98 (0.93-1.03)
35-55 years	1332	68420	1.11 (1.04-1.18)	1.11 (1.04-1.18)
White Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	2117	120709	1.17 (1.1-1.25)	1.17 (1.09-1.25)
25-29 years	1750	113375	Reference	Reference
30-34 years	1025	67397	0.97 (0.90-1.05)	0.96 (0.89-1.04)
35-55 years	404	24202	1.07 (0.95-1.19)	1.04 (0.93-1.16)
Asian Pacific Islander Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	236	14427	1.12 (0.94-1.34)	1.12 (0.93-1.35)
25-29 years	253	16649	Reference	Reference
30-34 years	198	13121	0.98 (0.81-1.18)	0.99 (0.82-1.19)
35-55 years	110	6914	1.06 (0.84-1.32)	1.07 (0.85-1.35)
Black Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	681	39256	0.87 (0.77-0.98)	0.89 (0.78-1.01)
25-29 years	443	21698	Reference	Reference
30-34 years	220	11514	0.89 (0.75-1.04)	0.90 (0.77-1.07)
35-55 years	105	4649	1.01 (0.81-1.25)	1.06 (0.85-1.32)
Hispanic Grandmother's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	3597	202948	0.99 (0.94-1.04)	0.99 (0.93-1.04)
25-29 years	2258	124560	Reference	Reference
30-34 years	1314	68724	1.04 (0.97-1.11)	1.05 (0.98-1.12)
35-55 years	713	32655	1.18 (1.08-1.28)	1.20 (1.10-1.31)

Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Table 3-S7. Model Fit by the Akaike Information Criterion for Intercept and Covariates

Grandmother's Age	**AIC**
Logistic Regression with Categorical Age (18-24, 25-29, 30-34, 35-55 years)	276148.89
Logistic Regression using Restricted Cubic Splines with 4 knots by Percentiles	276142.91
Logistic Regression using Restricted Cubic Splines with 5 knots by Percentiles	276142.10
Logistic Regression using Restricted Cubic Splines with 6 knots by Percentiles	276144.63
Grandfather's Age	**AIC**
Logistic Regression with Categorical Age (18-24, 25-29, 30-34, 35-55 years)	255515.44
Logistic Regression using Restricted Cubic Splines with 4 knots by Percentiles	255498.73
Logistic Regression using Restricted Cubic Splines with 5 knots by Percentiles	255500.63
Logistic Regression using Restricted Cubic Splines with 6 knots by Percentiles	255501.26

Note: All models are adjusted for offspring birth year (continuously scaled), offspring sex (male, female), mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), and grandparent's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Table 3-S8. Maternal Grandfather's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity

	Children, n		Odds Ratio (95% CI)			
Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 2**	**Model 3A**	**Model 4A**
18-24 years	7539	466935	1.07 (1.04-1.11)	1.04 (1.01-1.08)	1.04 (1.01-1.08)	1.03 (1.00-1.07)
25-29 years	8212	533729	Reference	Reference	Reference	Reference
30-34 years	5685	366087	0.99 (0.96-1.03)	1.01 (0.98-1.05)	1.01 (0.98-1.05)	1.02 (0.98-1.06)
35-55 years	4380	263971	1.06 (1.02-1.10)	1.08 (1.04-1.12)	1.08 (1.04-1.12)	1.08 (1.03-1.12)
White Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**	**Model 4B**
18-24 years	1943	123173	1.20 (1.13-1.27)	1.18 (1.11-1.25)	1.18 (1.11-1.25)	1.12 (1.05-1.20)
25-29 years	2534	190427	Reference	Reference	Reference	Reference
30-34 years	1957	144226	1.02 (0.96-1.08)	1.02 (0.96-1.08)	1.02 (0.96-1.08)	1.04 (0.98-1.11)
35-55 years	1366	97115	1.05 (0.98-1.12)	1.04 (0.98-1.12)	1.05 (0.98-1.12)	1.05 (0.97-1.14)
Asian Pacific Islander Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**	**Model 4B**
18-24 years	171	13054	0.89 (0.74-1.07)	0.87 (0.72-1.04)	0.87 (0.72-1.04)	0.87 (0.71-1.06)
25-29 years	351	23304	Reference	Reference	Reference	Reference
30-34 years	344	24463	0.92 (0.79-1.07)	0.93 (0.80-1.08)	0.93 (0.80-1.08)	0.95 (0.81-1.12)
35-55 years	298	21744	0.90 (0.77-1.05)	0.91 (0.77-1.06)	0.91 (0.77-1.07)	0.92 (0.76-1.12)
Black Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**	**Model 4B**
18-24 years	886	56132	0.93 (0.85-1.03)	0.91 (0.83-1.01)	0.91 (0.83-1.01)	0.93 (0.84-1.03)
25-29 years	790	46410	Reference	Reference	Reference	Reference
30-34 years	486	29194	0.97 (0.86-1.08)	0.99 (0.88-1.11)	0.99 (0.88-1.11)	1.00 (0.89-1.13)
35-55 years	492	25056	1.14 (1.01-1.27)	1.18 (1.05-1.32)	1.18 (1.05-1.32)	1.20 (1.05-1.36)
Hispanic Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 3B**	**Model 4B**

18-24 years	4539	274576	1.01 (0.97-1.05)	1.00 (0.96-1.05)	1.00 (0.96-1.05)	1.01 (0.97-1.06)
25-29 years	4537	273588	Reference	Reference	Reference	Reference
30-34 years	2898	168204	1.03 (0.99-1.08)	1.04 (0.99-1.09)	1.04 (0.99-1.09)	1.03 (0.98-1.08)
35-55 years	2224	120056	1.11 (1.05-1.17)	1.12 (1.06-1.18)	1.12 (1.06-1.18)	1.10 (1.03-1.16)

Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandfather's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Model 3A adjusts for the variables in model 2 plus mother's age (18-24, 25-29, 30-34, 35-55 years).

Model 4A adjusts for the variables in model 2 plus grandmother's age (18-24, 25-29, 30-34, 35-55 years).

Model 3B adjusts for the variables in model 1 plus mother's age (18-24, 25-29, 30-34, 35-55 years).

Model 4B adjusts for the variables in model 1 plus grandmother's age (18-24, 25-29, 30-34, 35-55 years).

Table 3-S9. Maternal Grandfather's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity, with Grandmother's Education Information

	Children, n		Odds Ratio (95% CI)	
Grandfather's Age	**Cases**	**Total**	**Model 2**	**Model 3A**
18-24 years	3619	206481	1.03 (0.98-1.08)	1.02 (0.97-1.07)
25-29 years	3598	212926	Reference	Reference
30-34 years	2483	145790	1.02 (0.97-1.07)	1.03 (0.98-1.09)
35-55 years	2011	109066	1.11 (1.05-1.17)	1.13 (1.07-1.20)
White Grandfather's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	732	41106	1.13 (1.02-1.25)	1.09 (0.99-1.21)
25-29 years	850	55171	Reference	Reference
30-34 years	674	44291	0.99 (0.89-1.10)	1.01 (0.91-1.12)
35-55 years	500	31984	1.02 (0.91-1.14)	1.05 (0.94-1.18)
Asian Pacific Islander Grandfather's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	81	5118	0.97 (0.73-1.31)	0.98 (0.73-1.31)
25-29 years	108	6607	Reference	Reference
30-34 years	88	5995	0.89 (0.67-1.19)	0.90 (0.68-1.20)
35-55 years	96	6154	0.95 (0.72-1.26)	0.96 (0.72-1.28)
Black Grandfather's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	406	24359	0.91 (0.79-1.06)	0.91 (0.79-1.06)
25-29 years	343	19146	Reference	Reference
30-34 years	229	12924	1.01 (0.86-1.20)	1.01 (0.86-1.20)
35-55 years	226	11255	1.16 (0.97-1.37)	1.15 (0.97-1.37)
Hispanic Grandfather's Age	**Cases**	**Total**	**Model 1**	**Model 3B**
18-24 years	2400	135898	1.02 (0.96-1.08)	1.01 (0.95-1.07)
25-29 years	2297	132002	Reference	Reference
30-34 years	1492	82580	1.05 (0.98-1.12)	1.05 (0.99-1.13)
35-55 years	1189	59673	1.16 (1.07-1.24)	1.17 (1.09-1.26)

Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female), mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandfather's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Model 3A adjusts for the variables in model 2 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more).

Model 3B adjusts for the variables in model 1 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more).

Table 3-S10. Maternal Grandfather's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity, without Grandfather's Age Restriction ≤ 55 Years

	Children, n		Odds Ratio (95% CI)
Grandfather's Age	**Cases**	**Total**	**Model 2**
25-29 years	8212	533729	Reference
≥35 years	4436	267648	1.07 (1.03-1.12)
White Grandfather's Age	**Cases**	**Total**	**Model 1**
25-29 years	2534	190427	Reference
≥35 years	1371	97961	1.04 (0.97-1.11)
Asian Pacific Islander Grandfather's Age	**Cases**	**Total**	**Model 1**
25-29 years	351	23304	Reference
≥35 years	304	22143	0.91 (0.77-1.06)
Black Grandfather's Age	**Cases**	**Total**	**Model 1**
25-29 years	790	46410	Reference
≥35 years	510	25973	1.18 (1.05-1.32)
Hispanic Grandfather's Age	**Cases**	**Total**	**Model 1**
25-29 years	4537	273588	Reference
≥35 years	2251	121571	1.12 (1.06-1.18)

Model 1 adjusts for offspring birth year (continuously scaled), offspring sex (male, female), mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandfather's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Table 3-S11. Maternal Grandfather's Age and Autism Spectrum Disorders in Grandchildren by Race/Ethnicity, among Firstborn Grandchildren

	Children, n		Odds Ratio (95% CI)	
Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 2**
18-24 years	3910	223394	1.06 (1.02-1.11)	1.05 (1.00-1.09)
25-29 years	4555	269396	Reference	Reference
30-34 years	3252	194112	0.97 (0.92-1.01)	0.98 (0.93-1.02)
35-55 years	2538	140780	1.03 (0.98-1.08)	1.04 (0.99-1.09)
White Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	1080	61057	1.19 (1.10-1.29)	1.18 (1.09-1.28)
25-29 years	1533	100765	Reference	Reference
30-34 years	1223	81258	0.98 (0.91-1.06)	0.98 (0.90-1.05)
35-55 years	865	55971	1.00 (0.92-1.08)	0.98 (0.90-1.07)
Asian Pacific Islander Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	80	6538	0.70 (0.54-0.91)	0.68 (0.52-0.88)
25-29 years	226	12604	Reference	Reference
30-34 years	224	14086	0.86 (0.71-1.04)	0.88 (0.73-1.06)
35-55 years	180	12273	0.80 (0.66-0.97)	0.82 (0.67-1.00)
Black Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	467	27595	0.89 (0.78-1.01)	0.90 (0.79-1.03)
25-29 years	454	23623	Reference	Reference
30-34 years	295	15324	0.98 (0.84-1.13)	0.98 (0.85-1.14)
35-55 years	279	12990	1.07 (0.92-1.25)	1.09 (0.94-1.27)
Hispanic Grandfather's Age	**Cases**	**Total**	**Model 0**	**Model 1**
18-24 years	2283	128204	1.02 (0.96-1.08)	1.02 (0.97-1.09)
25-29 years	2342	132404	Reference	Reference
30-34 years	1510	83444	1.01 (0.95-1.08)	1.02 (0.95-1.09)
35-55 years	1214	59546	1.14 (1.06-1.22)	1.15 (1.07-1.24)

Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female).

Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled) and grandmother's parity (1, 2, 3 or more).

Model 2 adjusts for the variables in model 1 plus grandfather's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Table 3-S12. Mother, Grandmother, and Grandfather's Age Correlation

	Sample Size	**Comparison**	**Spearman Correlation Coefficient (r)**
Grandmother's Age Analysis	1743998	Mother's vs Grandmother's Age Group	0.095
Grandfather's Age Analysis	1630722	Mother's vs Grandmother's Age Group	0.096
	1630722	Mother's vs Grandfather's Age Group	0.092
	1630722	Grandmother's vs Grandfather's Age Group	0.645

Table 3-S13. Mother's Age Distribution by Autism Spectrum Disorders

	Multigenerational Study Population		**2001-2019 California Birth Cohort**	
Mother's Age (in years)	Case (N=27975)	Total (N=1743998)	Case (N=117459)	Total (N=8625256)
Continuous, mean (SD)	25.0 (4.23)	24.5 (4.35)	29.6 (6.05)	28.9 (5.98)
Categorical, n (%)				
18-24 years	13568 (48.5%)	931681 (53.4%)	26696 (22.7%)	2302588 (26.7%)
25-29 years	9720 (34.7%)	541524 (31.1%)	31164 (26.5%)	2350223 (27.2%)
30-34 years	4429 (15.8%)	254130 (14.6%)	32473 (27.6%)	2335642 (27.1%)
35-55 years*	258 (0.9%)	16663 (1.0%)	27126 (23.1%)	1636803 (19.0%)

*In the multigenerational study population, the upper age limit is 36 instead of 55 years.

Table 3-S14. Mother's Demographics

	Full 2001-2019 California Birth Cohort				Multigenerational Birth Cohort
	Total	**Not CA Born**	**CA Born**		**Total**
	(N=9,803,024)	**(N=5,187,256)**	**(N=4,615,768)**		**(N=1,743,998)**
Mother's Race and Ethnicity, n (%)					
Asian Pacific Islander	1288182 (13.4%)	1095885 (21.6%)	192297 (4.2%)		72221 (4.2%)
Black	513851 (5.3%)	134385 (2.6%)	379466 (8.4%)		138143 (8.1%)
Hispanic of any race	4845406 (50.4%)	2700597 (53.2%)	2144809 (47.3%)		936808 (54.8%)
White	2737412 (28.5%)	1079495 (21.3%)	1657917 (36.6%)		505508 (29.6%)
Other	224646 (2.3%)	69111 (1.4%)	155535 (3.4%)		57266 (3.3%)
Missing	193527	107783	85744		34052
Mother's Nativity, n (%)					
US-born	5681546 (58.0%)	1065778 (20.6%)	4615768 (100.0%)		1743998 (100.0%)
Foreign-born	4109378 (42.0%)	4109378 (79.4%)	0 (0.0%)		
Missing	12100	12100	0		
Mother's Education, n (%)					
8th Grade or Less	739228 (7.8%)	702831 (14.1%)	36397 (0.8%)		9334 (0.6%)
9th to 12th Grade (No Diploma)	1444526 (15.3%)	816273 (16.4%)	628253 (14.1%)		232649 (13.8%)
High School Graduate	2467779 (26.1%)	1070174 (21.5%)	1397605 (31.3%)		621294 (36.9%)
Some Degree Less than College	2234136 (23.7%)	866528 (17.4%)	1367608 (30.6%)		574030 (34.1%)
College or More	2556057 (27.1%)	1515366 (30.5%)	1040691 (23.3%)		248053 (14.7%)
Missing	361298	216084	145214		58638

Figure 3-S1. Study Population Flowchart

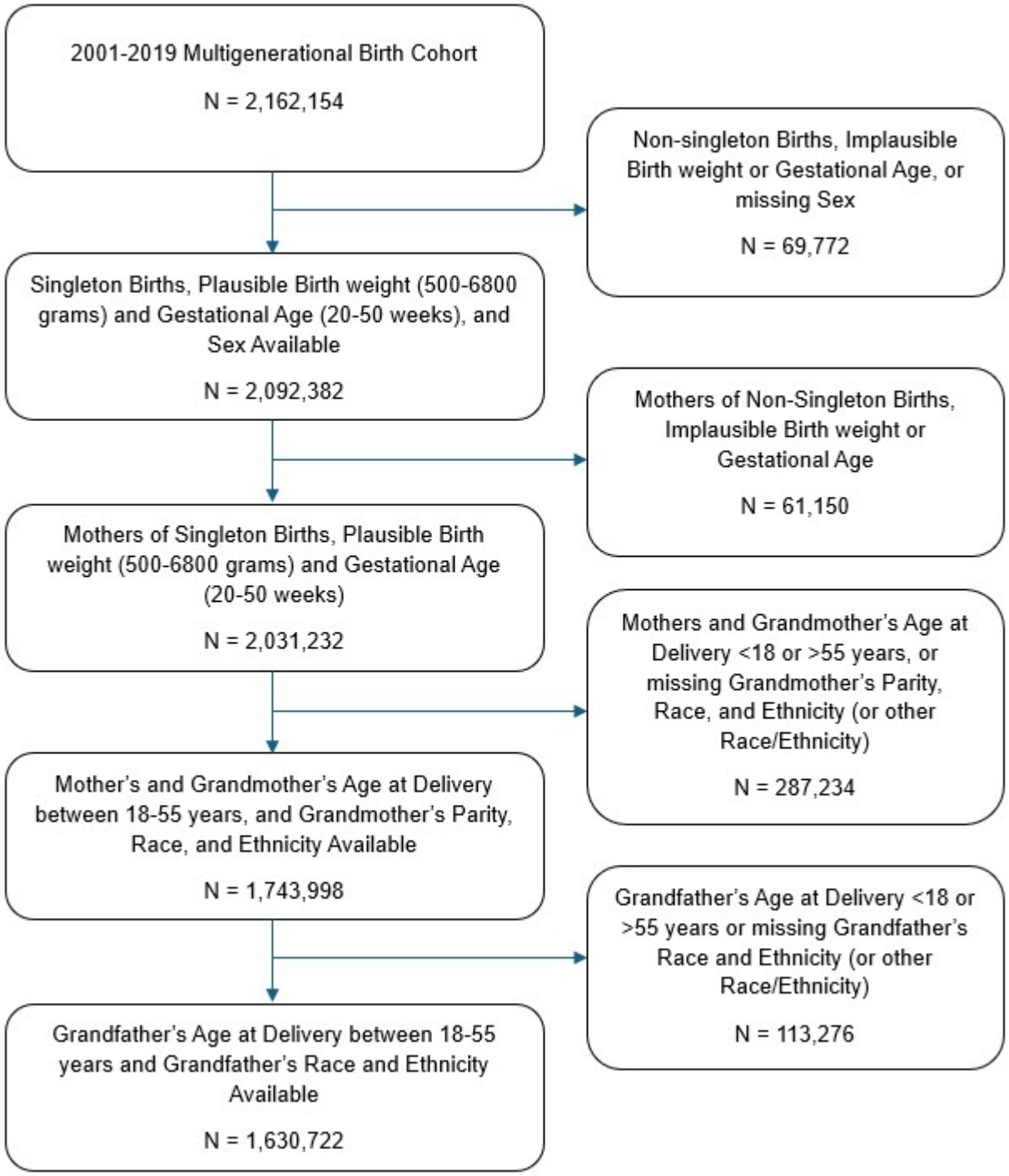
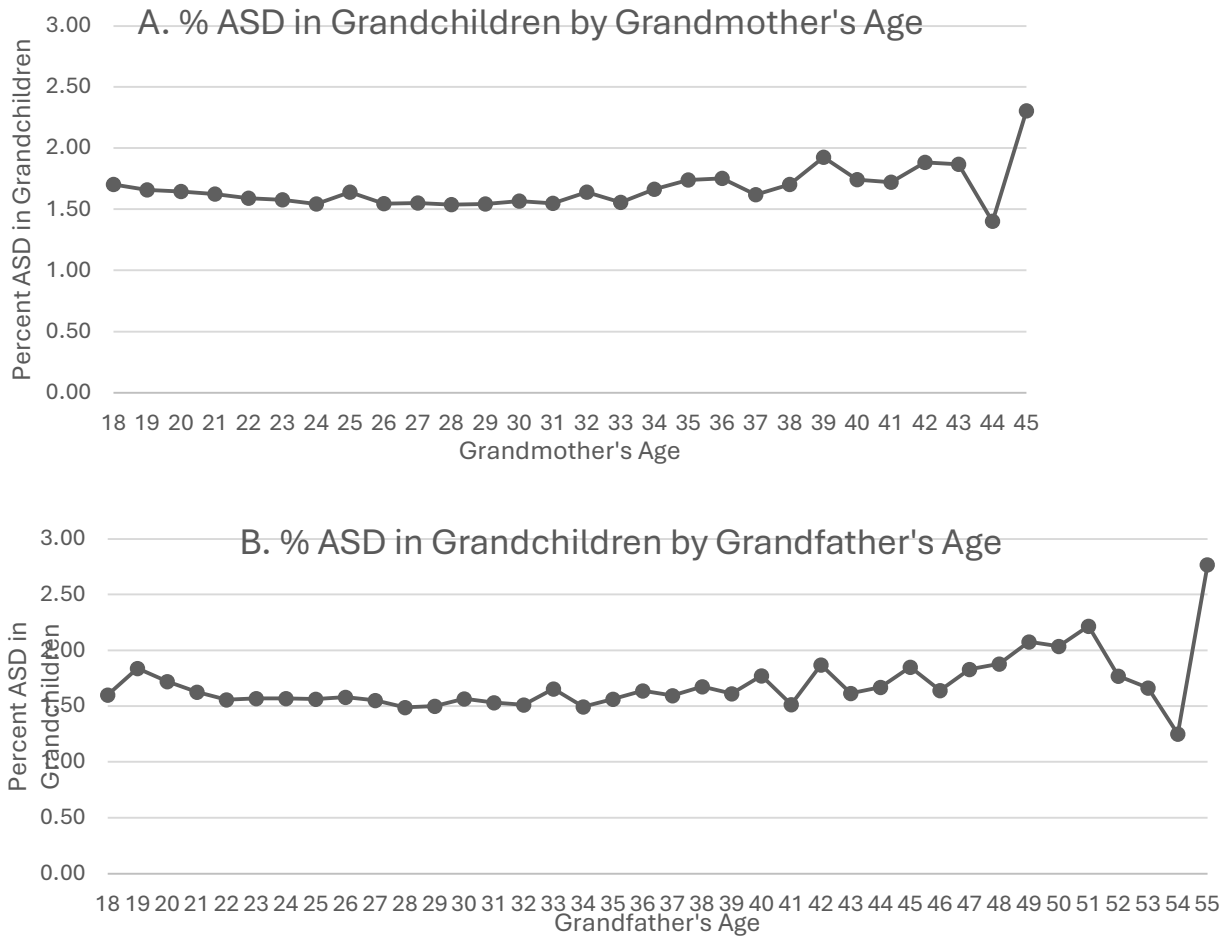


Figure 3-S2. Crude Percent of Autism Spectrum Disorders in Grandchildren by Maternal Grandparent's Age (1 Year Age Categories)



Note: Maternal grandmother's age was truncated at 45 years of age for this graph because the number of grandchildren with autism spectrum disorders was < 5 for grandmothers 46 years and older. ASD = autism spectrum disorders.

4. Autism Spectrum Disorders and Grandmother's Pregnancy Complications

4.1. Introduction

Autism spectrum disorders (ASD) is a neurodevelopmental disorder mainly defined by difficulties in social communication, repetitive behaviors, and restricted interests manifesting in early childhood [2,86]. The estimated prevalence of ASD in 2020 was 27.6 per 1000 children in the United States and 44.9 per 1000 children in California (among children born in 2012) according to the Centers of Disease Control and Prevention's (CDC) Autism and Developmental Disabilities Monitoring (ADDM) network [15]. While ASD is multifactorial, apart from genetics and older age at pregnancy, maternal risk factors for ASD include the mother's pregnancy complications including hypertensive disorders [29,30], infections [31], and diabetes [32]. In the United States, the rates of hypertensive disorders and diabetes in pregnancy have been increasing in the past decades [87]. For example, according to the CDC, the rates of chlamydia, gonorrhea, and syphilis in pregnancy increased by 2%, 16%, and 34% respectively from 2016 to 2018 [88].

Pregnancy complications may lead to suboptimal in-utero environments that may impair fetal neurodevelopment. Maternal hypertensive disorders and diabetes are thought to lead to placental dysfunction, fetal hypoxia, and/or increased oxidative stress [30,32]. Infections in pregnancy may affect offspring neurodevelopment through actions of the pathogen themselves or triggering a harmful immune response of the mother to the pathogen [31]. Maternal pregnancy complications may also lead to adverse birth outcomes [89-91], such as preterm birth (PTB) and/or low birth weight (LBW), which in turn are risk factors for ASD [28].

There is increasing evidence for multigenerational disease transmission of ASD. Recent studies have reported elevated risks of ASD among children whose grandparents were of younger or older age [33-35] and whose grandmothers smoked while pregnant with the mothers [36]. In

addition, one study reported higher ASD risk among children whose mothers or fathers were born prematurely or were LBW [37]. Germ cell reprogramming is one proposed mechanism for multigenerational transmission of disease where epigenetic markers typically removed and reestablished in early fetal development are impaired or altered [39,42]. It has been hypothesized that maternal immune activation (MIA) may have multigenerational impacts through epigenetic modifications and germ cell-dependent inheritance [41].

A study conducted in a population of predominately European ancestry [37] reported an increased risk of ASD in grandchildren whose mothers or fathers were born prematurely or were LBW. However, less is known about multigenerational associations of pregnancy complications in the grandmother and ASD in grandchildren. Here, we investigate the associations between pregnancy complications (hypertensive disorders, infection, and diabetes) experienced by the grandmother and ASD in her grandchildren and assess whether these associations are mediated by grandmothers giving birth to their daughter (the mother's generation) prematurely. We hypothesize that among grandmothers who experienced hypertensive disorders, infections, or diabetes in pregnancy, grandchildren are at higher odds of ASD compared to grandmothers without these respective conditions. Additionally, we hypothesize that these associations are mediated by the mother's premature birth which may have disrupted her own development during the fetal growth period. Specifically, the hypothesis for the mediation analysis is that the risk of ASD in grandchildren would not have been higher among grandmothers with hypertensive disorders, infections, and diabetes during pregnancy with the mother's generation compared to grandmothers who did not have these conditions, had their daughters (the mother's generation) not been born preterm. The percentage of the total effect of grandmother's hypertension, infection, or diabetes with ASD in grandchildren through mother's preterm birth status will be greater than 0%.

4.2. Methods

Study Population

This is a California population-based multigenerational birth cohort study of children born between 2001-2019 to mothers who were born between 1983-2001. Briefly, ASD diagnostic records obtained from the California Department of Developmental Services (DDS) [7] and birth records obtained from the California Department of Public Health [53] were probabilistically linked (using National Program of Cancer Registries Registry Plus Link Plus Software [55]) based on child and parental identifiers as previously described [1]. The DDS Client Developmental Evaluation Report (CDER) includes primary diagnosis of autistic disorder according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised (DSM-IV-R) until 2014 or DSM-V [6]. California born children with a linked ASD diagnostic record from the DDS were considered children with ASD and those whose birth record did not link with an ASD diagnostic record were considered non-cases.

Children and mothers were eligible for this study if they had been singleton births with plausible birth weight (500-6800 grams) and gestational age (20-50 weeks), mother's and grandmother's ages were 18-55 years, and information on child's sex, grandmother's parity, race, ethnicity, and pregnancy complications were available. The final analytical cohort includes 1,740,379 mother-child pairs with complete information for analysis. This study has been approved by the California State and UCLA Institutional Review Boards with waiver of consent.

Statistical Analysis

We first evaluated the associations between offspring ASD risk and grandmother's pregnancy complications i.e. when pregnant with the mother. We performed logistic regression to obtain odds ratios (ORs) and 95% CIs, adjusting for child's birth year and sex (male, female), mother's birth year, grandmother's parity (1, 2, 3 or more births), grandmother's age (18-24, 25-29, 30-

34, 35-55 years), and grandmother's race/ethnicity (Asian Pacific Islander (API), Black, Hispanic (any race), White). Covariates were included in the regression models as categorical (see above) or continuous (birth year). Grandmother's pregnancy complications (hypertensive disorders, infections, and diabetes in pregnancy) were recorded on maternal birth certificates as a string of 2-digit pregnancy complication codes (Table 4-S1). Grandmothers without the respective pregnancy complication were considered the reference group for each respective complication. All covariates and pregnancy complications were recorded on the mother or grandchild's birth certificates.

As sensitivity analyses, we additionally included grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more) and mother's birth weight (< 2500 grams, ≥ 2500 but < 4000 grams, ≥ 4000 grams) for the subset of mother-child pairs for whom the grandmother's educational level was available i.e. starting with 1989 birth records. These additional covariates are used as proxies to adjust for some of the confounding by grandmother's smoking status, which has been reported to be associated with ASD (adjusted OR = 1.41) [36] but was not collected on the California birth records until more recent birth years. A recent study found that five variables recorded on the California birth records (child's birth year, child's birth weight, mother's race/ethnicity, parity, and education) were predictive of mother's smoking in pregnancy with an overall discrimination accuracy of 0.72 and an area under the receiver operating characteristic curve of 0.81 [92]. We additionally report e-values to quantify the minimum strength an uncontrolled confounder (such as grandmother's smoking) would need to exhibit with both grandmother's pregnancy complications and ASD risk in grandchildren to fully explain away the associations we observed [93].

We then evaluated the associations between offspring ASD risk and the mother's adverse birth characteristics. The gestational week (according to the dates of last menses and of birth) when the mother was born was used to categorize preterm birth as being born < 37 weeks gestation. We also investigated a subset of very preterm birth defined as being born < 32 gestational weeks) and used term birth (37+ weeks of gestation) as the reference group for all preterm births. Mother's birth weight is categorized as LBW (< 2500 grams), normal weight (≥ 2500 but < 4000 grams), and macrosomia (≥ 4000 grams) and we will use normal birth weight as the reference group.

Finally, we additionally performed mediation analyses for mother's premature birth while estimating effects for each of the grandmother's pregnancy complications and ASD risk in grandchildren taking mother's preterm birth into account. Formally, casual mediation decomposition of the total effect (TE) requires identifiability (the stable unit treatment value assumption, consistency, conditional exchangeability, and positivity), and conditional exchangeability which requires (1) no uncontrolled confounding between the exposure and outcome, (2) no uncontrolled confounding between the mediator and outcome, (3) no uncontrolled confounding between the exposure and mediator, and (4) no exposure-induced confounding of the mediator and outcome [94]. To calculate the standard controlled direct effect (CDE), conditional exchangeability requirements (1) and (2) are sufficient.

Logistic regression was used to estimate the total effect between offspring ASD and each of the grandmother's pregnancy complications and to estimate the controlled direct effect had mothers not been preterm. The portion eliminated (PE) will equal the estimated total effect minus the estimated controlled direct effect. The PE divided by the estimated total effect will equal the proportion of the estimated effect of grandmother's pregnancy complication on ASD in grandchildren that is due to mediation by mother's having been preterm, the interaction between

grandmother's pregnancy complication and mother's having been preterm, or both the mediation and the interaction. The corresponding bootstrap percentile 95% CIs were generated using 1000 bootstrap samples. To preserve statistical power, we categorized the child's (2001-2009, 2010-2013, 2014-2016, 2017-2019) and mother's (1983-1985, 1986-1988, 1989-1991, 1992-2001) birth years and adjusted them using continuous variables. In a sensitivity analysis, we also instead relied on mothers very preterm status as the mediator since very premature birth is a more severe adverse birth outcome.

Data analysis was performed using SAS 9.4 (SAS Institute Inc.) and e-values using the online calculator [93].

4.3. Results

Our multigenerational study population included 1,740,379 mother-child pairs among whom 27,904 (1.6%) children were diagnosed with ASD (Table 4-1). While 51.1% of this birth cohort consisted of male children, 78.5% of the ASD cases were male children. About one third (31.8%) of the mothers were the firstborn daughters of the grandmothers and most grandmothers had given birth to their daughter by the age of 30 years. A majority of grandmothers were of Hispanic ethnicity of any race (50.9%) followed by grandmothers of White race (35.1%).

The odds of ASD differed by grandmother's pregnancy complications after adjusting for the child's birth year and sex, mother's birth year, grandmother's parity, age, and race/ethnicity (Table 4-2). The odds of ASD were higher among grandmothers who had experienced pregnancy hypertensive disorders (OR, 1.23; 95% CI, 1.12-1.36) or infections (OR, 1.14; 95% CI, 1.01-1.28) but not diabetes (OR, 1.06; 95% CI, 0.94-1.19) compared to grandmothers who did not suffer from the respective condition in pregnancy. Additional adjustment for

grandmother's education and mother's birth weight did not change the results (Table 4-S2). The e-values to fully explain away the associations observed between grandmother's hypertensive disorders or infections in pregnancy with ASD in grandchildren are 1.76 and 1.54 respectively (Table 4-S3).

The odds of ASD also differed by mother's adverse birth characteristics after adjusting for the child's birth year and sex, mother's birth year, grandmother's parity, age, and race/ethnicity (Table 4-2). The odds of ASD were higher among mothers born prematurely (OR, 1.06; 95% CI, 1.02-1.11) and very prematurely (OR, 1.19; 95% CI, 1.07-1.32) compared to mothers being born at term, respectively. The odds of ASD were similar among mothers born with a macrosomic birth weight (OR, 1.02; 95% CI, 0.97-1.06) and slightly elevated among mothers born with LBW (OR, 1.05; 95% CI, 1.00-1.11) compared to mothers with normal birth weight, respectively.

We did not observe mediating effects by mothers born prematurely on the associations between grandmother's hypertensive disorders or infection and ASD in grandchildren (Table 4-S4).

4.4. Discussion

In this large and diverse California birth cohort covering almost 40 years, we observed that the risk of ASD in grandchildren is elevated among grandmothers with hypertensive disorders or infections in pregnancy compared to grandmothers who did not suffer from these respective pregnancy complications. With the rising prevalence of hypertensive disorders and infection in pregnancy [87,88], these results are of public health importance as they suggest these pregnancy complications may affect neurodevelopmental outcomes two generations later.

Results remained essentially the same even after adjusting for grandmother's education and mother's birth weight or preterm status. However, grandmother's smoking is a potential

confounder that we could not directly adjust. A study from the United Kingdom reported an association between grandmother's smoking in pregnancy and ASD in grandchildren (adjusted OR = 1.41) [36]. Yet, the calculated e-values compared with the estimate of grandmother's smoking and ASD risk in grandchildren previously reported indicate that confounding by grandmother's smoking is unlikely to fully explain away the associations of grandmother's hypertensive disorders or of infections with ASD occurrence in the grandchildren. While there is some evidence that maternal smoking may be related to maternal hypertensive disorders in pregnancy [95], it is less likely that maternal smoking would be related to maternal infections. Therefore, grandmother's smoking might not be a confounder between the association of grandmother's infection in pregnancy and ASD risk in grandchildren. In addition, the majority of grandmothers in our California multigenerational cohort are Hispanics of any race. A study of children born in Los Angeles County found that Latina mothers reported very low smoking rates - lower than White mothers [96]. However, there may still be residual confounding from other unmeasured potential confounders such as grandmother's alcohol drinking or mental health.

While the association between grandmother's diabetes in pregnancy and ASD in grandchildren was in a positive direction, it might not have reached statistical significance since there was both a small number of grandmothers with diabetes who also had a grandchild with ASD and the magnitude of the OR was weak. We therefore did not attempt mediation analyses for grandmother's diabetes in pregnancy. Future research with a larger number of grandmothers with diabetes, data to differentiate between different types of diabetes (e.g. gestational, type 1, or type 2), glucose control in pregnancy, and pre-pregnancy body mass index are needed to better understand potential risk and mechanisms involved.

There is increasing evidence from animal models that maternal immune activation in pregnancy may have multigenerational effects [41]. In murine models, exposure to polyinosinic:polycytidylic

acid (poly(I:C)), an immunostimulant to mimic viral immune activation, in early pregnancy resulted in reduced sociability and increased fear-related behaviors in the subsequent three generations [97] and in mid pregnancy this exposure resulted in increased depressive-like behaviors and some increased hippocampal miRNA expression in the offspring two generations later [98].

Consistent with a Danish study, we found that ASD in grandchildren was increased among mothers born prematurely (or very prematurely) compared to mothers born at term and among mothers born LBW compared to mothers born with normal birth weight [37]. Proposed mechanisms for multigenerational transmission of disease includes fetal germ cell reprogramming, where disturbances disrupt the establishment of epigenetic marks in the developing fetal germline [39,42]. Therefore, we investigated whether grandmother's pregnancy complications influenced grandchildren's ASD risk and whether this risk was mediated by the mothers having been preterm infants (as an indicator of disrupted fetal development).

We did not detect any evidence for mediation of grandchildren's ASD risk according to grandmother's hypertensive disorders or infection by mothers' preterm status. The CDEs also appeared elevated compared to the TEs, indicating that the associations between grandmother's hypertensive disorders or infections with ASD in grandchildren are stronger among mothers born term. A possible explanation for these results include collider stratification bias since the proportions eliminated were in the inverse direction and generally null, which was contrary to expectations and reminiscent of the 'birth weight paradox' seen in the association between maternal smoking and lower infant mortality [99]. Using preterm birth as a mediator may have induced collider bias introduced through an unknown confounder of the association between maternal preterm birth and ASD in offspring. Specifically, if there are unmeasured common causes between mother's preterm birth and ASD in grandchildren, conditioning on

mother's preterm birth may open a backdoor path between grandmother's pregnancy complications and ASD in grandchildren. When we instead used very preterm birth as the mediator (more severe and perhaps better indicator of fetal developmental disturbance), we found that the proportion eliminated was reduced to null, especially for grandmother's infection.

In addition, while it was more common that mothers were born prematurely than mothers having had LBW (Table 2), preterm status may not be a good or very specific indicator or proxy for maternal fetal developmental disturbances that affect epigenetic markers. Other possible explanations include a lack of statistical power due to the small number of grandmothers who had suffered from each of the pregnancy complications and had a grandchild with ASD as well as the relatively small number of mothers born preterm with a child with ASD. This is reflected in the wide 95% CIs for the proportions eliminated that for the most part crossed the null value of 0%. Like with any birth cohort, there may also be live birth bias [100] which may occur if the daughters of exposed grandmothers (with pregnancy complications) who would also have been at risk for being born preterm are more likely to not be born alive.

Strengths of this study include its California wide coverage and its ethnically diverse population, a relatively large sample size, and pregnancy complications having been recorded on the birth records eliminating recall bias since grandmother's information was recorded before the grandchild's ASD diagnosis. All children with or without ASD were also included in the analytical study population according to the same eligibility criteria. In addition, there is a high specificity (low false positive rate) of ASD since assessments are made by well-trained multidisciplinary teams at the DDS [11]. However, growing awareness of ASD may have influenced the sensitivity of ASD over the years, so we added birth year into the covariate adjustment.

However, despite our large sample size, we still may have lacked statistical power in the analysis of grandmother's diabetes and for detecting mediation. Alternatively, there may have been live birth bias both in the grandmother-mother and mother-child generation and/or collider bias introduced in the mediation analyses. In addition, we were unable to adjust for grandmother's smoking but performed additional sensitivity analyses and did not have sufficient sample size and/or detailed data to look at specific types of hypertensive disorders, infections, or diabetes. These pregnancy complications were also self-reported and therefore may have been misclassified; however, this misclassification is unlikely to be related to ASD in grandchildren but may have resulted in an attenuation of our effect sizes. Mothers in our study population also had to be born in California, stay in California, and give birth in California. California birth certificate data became available electronically in 1983, therefore by default maternal age was younger. Future studies with longer follow-up are needed to confirm these findings. Nationwide studies may also be needed to capture all mothers who left California except for those who emigrated from the United States. Selection bias induced by moving out of California may however be minimal if moving is not strongly related to pregnancy complications or ASD outcome. We also do not have epigenetic data from the mother of the ASD child to examine the hypothesis of epigenetic reprogramming.

4.5. Conclusion

Our findings suggest that there may be multigenerational impacts of pregnancy complications in grandmothers that increase the risk of the grandchildren developing ASD. These findings are important since pregnancy complications such as hypertensive disorders are common in the obstetric population and the rates are rising. However, future studies to confirm these findings are needed.

4.6. Tables

Table 4-1. Study Population Characteristics by Autism Spectrum Disorders

Study Population Characteristics	Case (N=27904)	Total (N=1740379)
Child's Sex, n (%)		
Female	6004 (21.5%)	851199 (48.9%)
Male	21900 (78.5%)	889180 (51.1%)
Mother's Birth Year, mean (SD)	1988.6 (4.00)	1988.3 (3.94)
Grandmother's Parity, n (%)		
1	9067 (32.5%)	553109 (31.8%)
2	8407 (30.1%)	538310 (30.9%)
3 or more	10430 (37.4%)	648960 (37.3%)
Grandmother's Race and Ethnicity, n (%)		
Asian Pacific Islander	1297 (4.6%)	92390 (5.3%)
Black	2611 (9.4%)	151513 (8.7%)
Hispanic	15203 (54.5%)	886413 (50.9%)
White	8793 (31.5%)	610063 (35.1%)
Grandmother's Age Categories, n (%)		
18-24 years	12627 (45.3%)	781853 (44.9%)
25-29 years	8354 (29.9%)	534252 (30.7%)
30-34 years	4724 (16.9%)	297673 (17.1%)
35-55 years	2199 (7.9%)	126601 (7.3%)
Grandmother's Hypertensive Disorders in Pregnancy, n (%)		
Yes	435 (1.6%)	21953 (1.3%)
Grandmother's Infections in Pregnancy, n (%)		
Yes	293 (1.1%)	15131 (0.9%)
Grandmother's Diabetes in Pregnancy, n (%)		
Yes	293 (1.1%)	16480 (0.9%)
Mother's Birthweight Categories, n (%)		
Normal birthweight (>=2500g but <4000g)	24035 (86.1%)	1503914 (86.4%)
Macrosomia (>=4000g)	2491 (8.9%)	154787 (8.9%)
Low birthweight (<2500g)	1378 (4.9%)	81678 (4.7%)
Mother's Gestational Age Categories, n (%)		
Non-preterm (>=37 weeks)	25330 (90.8%)	1588935 (91.3%)
Preterm (<37 weeks)	2574 (9.2%)	151444 (8.7%)

Table 4-2. Grandmother's Pregnancy Complications and Autism Spectrum Disorders in Grandchildren

	Children, n		Odds Ratios (95% CI)	
Grandmother's Hypertensive Disorders in Pregnancy	**Cases**	**Total**	**Model 0**	**Model 1**
No	27469	1718426	Reference	Reference
Yes	435	21953	1.24 (1.13-1.36)	1.23 (1.12-1.36)
Grandmother's Infections in Pregnancy	**Cases**	**Total**	**Model 0**	**Model 1**
No	27611	1725248	Reference	Reference
Yes	293	15131	1.15 (1.02-1.29)	1.14 (1.01-1.28)
Grandmother's Diabetes in Pregnancy	**Cases**	**Total**	**Model 0**	**Model 1**
No	27611	1723899	Reference	Reference
Yes	293	16480	1.07 (0.95-1.20)	1.06 (0.94-1.19)
Mothers Born Premature	**Cases**	**Total**	**Model 0**	**Model 1**
Non-preterm (>=37 weeks)	25330	1588935	Reference	Reference
Preterm (<37 weeks)	2574	151444	1.08 (1.04-1.13)	1.06 (1.02-1.11)
Very preterm (<32 weeks)	354	18459	1.22 (1.10-1.36)	1.19 (1.07-1.32)
Mothers Birthweight	**Cases**	**Total**	**Model 0**	**Model 1**
Normal birthweight (>=2500g but <4000g)	24035	1503914	Reference	Reference
Macrosomia (>=4000g)	2491	154787	1.01 (0.96-1.05)	1.02 (0.97-1.06)
Term macrosomia (>=4000g and >=37 weeks)	2397	149018	1.00 (0.96-1.05)	1.02 (0.97-1.06)
Low birthweight (<2500g)	1378	81678	1.07 (1.01-1.13)	1.05 (1.00-1.11)
Term low birthweight (<2500g and >=37 weeks)	720	43355	1.06 (0.98-1.14)	1.04 (0.97-1.12)

Note: Very preterm is a subset of preterm, term macrosomia is a subset of macrosomia, term low birth weight is a subset of low birth weight.
Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female participants).
Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), grandmother's age (18-24, 25-29, 30-34, 35-55 years) and grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

4.7. Supplemental Materials

Table 4-S1. Hypertensive Disorders, Infections, and Diabetes in Pregnancy Codes from 1983-2001 Birth Records

	Mother's Birth Year																		
	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
Hypertensive Disorders																			
Pre-eclampsia/Eclampsia/Toxemia	x	x	x	x	x	x													
Pre-eclampsia/Pregnancy-induced hypertension							x	x	x	x	x	x	x	x	x	x	x	x	x
Eclampsia							x	x	x	x	x	x	x	x	x	x	x	x	x
Chronic hypertension							x	x	x	x	x	x	x	x	x	x	x	x	x
Infections																			
Urinary Tract/Kidney Infection	x	x	x	x	x	x													
Syphilis	x	x	x	x	x	x													
Rubella	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hepatitis B							x	x	x	x	x	x	x	x	x	x	x	x	x
Kidney infection/Pyelonephritis							x	x											
Pyelonephritis									x	x	x	x	x	x	x	x	x	x	x
Genital herpes									x	x	x	x	x	x	x	x	x	x	x
Other sexually transmitted disease									x	x	x	x	x	x	x	x	x	x	x
Diabetes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table 4-S2. Grandmother's Pregnancy Complications and Autism Spectrum Disorders in Grandchildren, among mothers born 1989-2001 and non-missing Grandmother's Education

	Children, n		Odds Ratios (95% CI)			
Grandmother's Hypertensive Disorders in Pregnancy	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 2**	**Model 3**
No	12983	737098	Reference	Reference	Reference	Reference
Yes	226	10525	1.22 (1.07-1.40)	1.22 (1.07-1.40)	1.22 (1.07-1.40)	1.21 (1.06-1.39)
Grandmother's Infections in Pregnancy	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 2**	**Model 3**
No	12973	736075	Reference	Reference	Reference	Reference
Yes	236	11548	1.14 (1.00-1.30)	1.16 (1.01-1.32)	1.16 (1.01-1.32)	1.16 (1.01-1.32)
Grandmother's Diabetes in Pregnancy	**Cases**	**Total**	**Model 0**	**Model 1**	**Model 2**	**Model 3**
No	13016	737617	Reference	Reference	Reference	Reference
Yes	193	10006	1.07 (0.93-1.24)	1.07 (0.92-1.23)	1.07 (0.92-1.23)	1.06 (0.92-1.23)

Model 0 adjusts for offspring birth year (continuously scaled) and offspring sex (male, female participants).

Model 1 adjusts for the variables in model 0 plus mother's birth year (continuously scaled), grandmother's parity (1, 2, 3 or more), grandmother's age (18-24, 25-29, 30-34, 35-55 years) and grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

Model 2 adjusts for the variables in model 1 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more) and mother's birth weight (< 2500 grams, ≥ 2500 but < 4000 grams, ≥ 4000 grams).

Model 3 adjusts for the variables in model 1 plus grandmother's education (8th grade or less, 9th to 12th but no diploma, high school, some degree less than college, college or more) and mother's premature birth (<37 weeks, >=37 weeks).

Table 4-S3. E-values to Explain away Grandmother's Pregnancy Complications and Autism Spectrum Disorders in Grandchildren

Pregnancy Complication	**Odds Ratios (95% CI)**	**E-value**
Grandmother's Hypertensive Disorders in Pregnancy	1.23 (1.12-1.36)	1.76
Grandmother's Infections in Pregnancy	1.14 (1.01-1.28)	1.54

Note: https://www.evalue-calculator.com/evalue/

Table 4-S4. Grandmother's Pregnancy Complications and Autism Spectrum Disorders in Grandchildren, by Mother's Premature Birth

		Odds Ratios (Bootstrap Percentile 95% CI)		
Grandmother's Pregnancy Complication	**Mother's Premature Birth**	**Total Estimated Effect**	**Estimated Controlled Direct Effect**	**Proportion Eliminated, % (95% CI)**
Grandmother's Hypertensive Disorders	Mothers Born Preterm (< 37 weeks)	1.23 (1.11-1.35)	1.24 (1.11-1.37)	-4.8 (-32.6,22.9)
Grandmother's Hypertensive Disorders	Mothers Born Very Preterm (< 32 weeks)	1.24 (1.10-1.37)	1.24 (1.11-1.38)	-2.2 (-13.1,10.0)
Grandmother's Infections	Mothers Born Preterm (< 37 weeks)	1.13 (1.01-1.27)	1.18 (1.05-1.33)	-34.2 (-188.1,-0.2)
Grandmother's Infections	Mothers Born Very Preterm (< 32 weeks)	1.18 (1.04-1.31)	1.18 (1.04-1.32)	-3.1 (-18.2,7.6)

Note: 95% CIs were obtained from 1000 bootstrap samples.

Associations are adjusted for offspring birth year (2001-2009, 2010-2013, 2014-2016, 2017-2019), offspring sex (male, female), mother's birth year (1983-1985, 1986-1988, 1989-1991, 1992-2001), grandmother's parity (1, 2, 3 or more), grandmother's age (18-24, 25-29, 30-34, 35-55 years) and grandmother's race/ethnicity (Asian Pacific Islander, Black, Hispanic, White).

The estimated controlled direct effect is the effect among mothers not born prematurely (>=37 weeks).

5. Public Health Relevance and Expected Contributions

This dissertation aimed to investigate the associations between multigenerational risk factors and ASD in grandchildren. Through a multigenerational California birth cohort, this dissertation confirms previous findings from European population-based studies and expanded on these findings in an ethnically and racially diverse population of California. These results add to the growing evidence of multigenerational disease transmission of ASD.

The first aim of this dissertation investigated the associations between grandmother's race, ethnicity, and nativity with ASD risk in grandchildren. Grandchildren of grandmothers who were foreign-born or US-born Black or Hispanic had higher odds of ASD than grandchildren of grandmothers who were US-born White. This adds to the current literature of multigenerational risk factors and ASD based on European study populations of predominately White race. These findings are also important since environmental exposures are unevenly distributed in California and may be a potential reason for this difference in grandchildren's ASD risk by grandmother's race, ethnicity, and nativity.

The second aim of this dissertation investigated the associations between maternal grandmother and grandfather's age and ASD in grandchildren, confirming previous findings. Grandparents of younger and older ages had higher or elevated odds of grandchildren with ASD. However, the higher risk of ASD in grandchildren observed for younger grandparents was only observed among grandparents who were of White race. These differences in the associations between ASD and grandparents' age by the grandparents' respective race/ethnicity may imply differing mechanisms for ASD risk among older and younger grandparents.

The third aim of this dissertation investigated the associations between grandmother's pregnancy complications (when pregnant with the mother's generation) and ASD in

grandchildren. Grandmothers who had pregnancy hypertensive disorders or infections (when pregnant with their daughters) had higher odds of grandchildren with ASD than grandmothers without these respective conditions. These findings are important since the prevalence of hypertensive disorders and infections have been increasing in the obstetric population and may have multigenerational impacts on neurodevelopment.

Strengths of this study include its California wide coverage in an ethnically and racially diverse population, prospective recording of the data (exposures and covariates when the grandmother is pregnant is recorded on the mother's birth certificate well before grandchildren's ASD assessment), and the diagnosis of ASD by the California DDS. However, limitations include the still lacking sample size in the API population, the truncation of mother's age due to data availability, and the lack of environmental, epigenetic, and more detailed health data.

In conclusion, the odds of ASD in grandchildren may be associated with grandmother's race, ethnicity, and nativity, with maternal grandparents' age, and with grandmother's hypertensive disorders or infections when pregnant with their daughters. These findings are important because they may imply that social and environmental exposures may affect the neurodevelopment of multiple subsequent generations. However, future studies with longer follow-up time and with social, environmental, occupational, and epigenetic data are needed to confirm and expand on these findings.

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