Epigenetics of Autism-related Impairment: Copy Number Variation and Maternal Infection

Varvara Mazina, BA,*† Jennifer Gerdts, PhD,† Sandy Trinh, MS,† Katy Ankenman, MSW,† Tracey Ward, BA,† Megan Y. Dennis, PhD,‡ Santhosh Girirajan, MBBS, PhD,‡§ Evan E. Eichler, PhD,‡‖ Raphael Bernier, PhD†

ABSTRACT: ***Objective:*** **Epidemiological data have suggested maternal infection and fever to be associated with increased risk of autism spectrum disorder (ASD). Animal studies show that gestational infections perturb fetal brain development and result in offspring with the core features of autism and have demonstrated that behavioral effects of maternal immune activation are dependent on genetic susceptibility. The goal of this study was to explore the impact of ASD-associated copy number variants (CNVs) and prenatal maternal infection on clinical severity of ASD within a dataset of prenatal history and complete genetic and phenotypic findings.** ***Methods:*** **We analyzed data from the Simons Simplex Collection sample including 1971 children with a diagnosis of ASD aged 4 to 18 years who underwent array comparative genomic hybridization screening. Information on infection and febrile episodes during pregnancy was collected through parent interview. ASD severity was clinically measured through parent-reported interview and questionnaires.** ***Results:*** **We found significant interactive effects between the presence of CNVs and maternal infection during pregnancy on autistic symptomatology, such that individuals with CNVs and history of maternal infection demonstrated increased rates of social communicative impairments and repetitive/restricted behaviors. In contrast, no significant interactions were found between presence of CNVs and prenatal infections on cognitive and adaptive functioning of individuals with ASD.** ***Conclusions:*** **Our findings support a gene-environment interaction model of autism impairment, in that individuals with ASD-associated CNVs are more susceptible to the effects of maternal infection and febrile episodes in pregnancy on behavioral outcomes and suggest that these effects are specific to ASD rather than to global neurodevelopment.**

*(J Dev Behav Pediatr 36:*61–67, 2015) **Index terms:** autism, autism spectrum disorders, fever, gene-environment, infection, pregnancy.

Autism spectrum disorder (ASD) has a strong genetic basis and converging lines of evidence implicate genetic factors as a predominant cause of ASD.[1] DNA microarrays enabled the discovery of rare and recurrent copy number variants (CNVs) as important contributors to ASD and led to rapid gains in the understanding of autism genetics and the identification of individuals genetically susceptible to autism.[2,3] Hot spots of recurrent CNVs, including those at 16p11.2, 22q11.2, 1q21.1, 7q11.23, and 15q11-q13, have been shown to be strongly associated with ASDs.[3–5]

A number of risk factors related to early fetal environment have been found in epidemiological research to be associated with increased rates of ASD.[6] Taken together, these environmental insults share in common the activation of the maternal immune system. Maternal immune activation (MIA) as a risk factor in ASD has been supported by a number of population-based cohort studies that found associations between autism risk and maternal viral infection in the first trimester, bacterial infection in the second trimester, and influenza and febrile episodes during pregnancy.[7,8] These epidemiological findings are supported by evidence from rodent and nonhuman primate model studies of autism-like behaviors in offspring after MIA induction by systemic administration of synthetic double-stranded RNA or lipopolysaccharide to mimic infection in the pregnant mother. Studies of offspring behavior after MIA have shown that gestational infections trigger a maternal immune response that can perturb fetal brain development,

From the *University of Washington School of Medicine, Seattle, WA; Departments of †Psychiatry, and ‡Genome Sciences, University of Washington, Seattle, WA; §Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA; ‖Howard Hughes Medical Institute, University of Washington, Seattle, WA.

Received September 2014; accepted November 2014.

Supported by 2 grants from the Simons Foundation (SFARI #89368 to R. Bernier and SFARI #137578 to E. E. Eichler) and a grant from the National Institute of Mental Health (#1R01MH101221 to E. E. Eichler); also supported by the Medical Student Research Training Program (MSRTP), University of Washington School of Medicine, Seattle, WA.

Disclosure: E. E. Eichler is an investigator of the Howard Hughes Medical Institute, is on the scientific advisory board (SAB) of DNAnexus, Inc., and was an SAB member of Pacific Biosciences, Inc. (2009–2013) and SynapDx Corp. (2011–2013). The remaining authors declare no conflict of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jdbp.org).

Address for reprints: Raphael Bernier, PhD, Center on Human Development and Disability, University of Washington, Box 357920, Seattle, WA 98195; e-mail: rab2@u.washington.edu.

by acting indirectly on the fetal brain chiefly through interleukin 6, and result in offspring with the core features of autism.[9,10] Although mounting evidence supports the connection between a strong immune response during gestation with risk of development of autistic traits, most mothers who develop an infection during pregnancy do not give birth to autistic children. The hypothesis that genetic susceptibility combined with a strong immune reaction during gestation results in increased autism severity and a greater likelihood of a diagnosis of ASD is supported by rodent model studies showing that offspring of mothers from an inbred strain predisposed to exhibit ASD-like behaviors were more severely affected after MIA than pups of a species typical strain, demonstrating that behavioral and immunological effects are strain dependent.[11] These findings also demonstrated evidence for genetic subtypes in ASD because of the differential behavioral profiles associated with gestational age and the onset of the environmental insult (e.g., MIA).[11] Furthermore, the combination of MIA and genetic susceptibility (CNVs) produces more severe behavioral profiles than either one of these factors alone, suggesting that the heterogeneity of behavioral phenotypes associated with ASD may have 3 distinct etiological contributions: genetic, environmental, and a combination of these factors.[11] In this study, we investigate this gene-environment interaction hypothesis by evaluating the interactive effects of maternal infection in pregnancy and the presence of CNVs considered likely to play a contributing role in symptoms of ASD (ASD-associated CNVs) using a dataset of parent-reported prenatal history and genetic and phenotypic findings.

MATERIALS AND METHODS

Study Population

Participants included 1971 children (1711 males, 260 females; 79% white, 89% non-Hispanic) with autism spectrum disorder (ASD) between the ages of 4 and 18 years (mean = 9.0, SD = 3.6) from the Simons Simplex Collection (SSC; see sfari.org). Individuals were selected for this analysis from the total sample of 2761 children with ASD if complete genetic, maternal pregnancy history, and phenotypic information was available.

The SSC is a project funded by the Simons Foundation Autism Research Initiative to identify de novo genetic variants related to ASD. Data were collected across 12 sites in North America. Inclusion criteria for the SSC required that the child meets the ASD clinical cutoffs on the Autism Diagnostic Observation Schedule (ADOS)[12] and on the Autism Diagnostic Interview-Revised (ADI-R)[13] as well as meets the diagnostic criteria for ASD using the Diagnostic and Statistical Manual of Mental Disorders (DSM-4-TR).[14] Probands were excluded for conditions that might jeopardize the validity of diagnostic instruments and for family history that might decrease likelihood of discovery of de novo events; a complete listing of inclusion and exclusion criteria can be found on www.sfari.org.[15]

This study was approved by the University of Washington institutional review board, and appropriate informed consent was obtained from all participants across all sites.

Genetic Analysis

Copy number variant (CNV) data were extracted from published array comparative genomic hybridization experiments on 2478 individuals from the SSC collection. These arrays were designed to identify recurrent CNVs or hot spots flanked by high identity repeat sequences including segmental duplications and nonrecurrent CNVs in the genomic backbone.[5] A dichotomous variable was used to indicate presence of any rare recurrent CNV previously identified to be enriched in individuals with autism. One hundred fifty-three individuals in our sample had at least 1 recurrent large hot spot event identified by Girirajan et al[5] (see Table, Supplemental Digital Content 1, http://links.lww.com/JDBP/A68, for a complete list of previously identified CNVs).

Maternal Infection and Febrile Episodes

Information on maternal infections and febrile episodes during pregnancy with the proband were collected as part of a larger medical history interview conducted with one or both parents (primarily mothers) during enrollment into the SSC. The specific infectious illnesses reported and the number of mothers who endorsed these infections during varying points of the pregnancy are outlined in Table 1. A dichotomous variable was created for history of maternal infection during the entire pregnancy as there was no significant difference between the onset of infection during pregnancy, and a similar pattern of results was seen regardless of trimester when the infection occurred.

Autism Presentation

Degree of autistic impairment for the probands for whom array comparative genomic hybridization results were available was determined through parent-reported measures of symptoms related to ASD: ADI-R, the Repetitive Behavior Scale-Revised (RBS-R), and the Social Responsiveness Scale (SRS). The ADI-R is a standardized semi-structured parent interview aligned with DSM-4 criteria used to aid in ASD diagnosis.[13] Three domains of the ADI-R were used as metrics of autistic symptomatology: Reciprocal Social Interaction, Communication, and Restricted, Repetitive, and Stereotyped Patterns of Behavior. The RBS-R is a parent-reported questionnaire regarding the presence and significance of restricted and repetitive behaviors that yields a total raw score summing 6 subscales: Stereotyped, Self-injurious, Compulsive, Routine, Sameness, and Restricted Behaviors.[16,17] The SRS is a 65-item rating scale assessing social awareness, social information processing, capacity for reciprocal social communication, social anxiety/avoidance, and autistic preoccupations and traits that yields a total T score using for quantitative measurement of ASD-related traits.[18]

Table 1. Reported Infectious Illnesses and Observed Copy Number Variants in Probands (N = 1971)

	No. of Exposed Probands with CNVs	No. of Exposed Probands Without CNVs	No. of Nonexposed Probands with CNVs	No. of Nonexposed Probands Without CNVs
Any infection or fever	29	366	124	1452
Other viral infection	10	154	141	1640
Maternal fever >101°F	10	63	141	1738
Influenza	7	94	144	1708
Herpes type 2 (HSV-2)	2	31	148	1770
Herpes type 1 (HSV-1)	1	37	150	1763
Chicken pox	1	3	150	1799
Shingles	1	1	150	1801
Viral hepatitis	0	3	151	1799
Infectious mononucleosis	0	2	151	1799
Measles	0	0	151	1802
Mumps	0	0	151	1802
Rubella	0	0	151	1802

CNV, copy number variant.

Higher scores across all measures suggest greater number of ASD-related behaviors, suggesting greater impairment in ASD symptoms.

In addition to parent-reported measures of the degree of autism-related symptoms, we also investigated the relationship between genetic susceptibility, maternal infections, and cognitive and adaptive functioning. Verbal and nonverbal IQ was measured using age-appropriate standardized assessment of cognitive functioning, including the Differential Abilities Scale-Second Edition (DAS-II),[19] the Wechsler Intelligence Scale for Children,[20] or the Mullen Scales of Early Learning.[21] Adaptive functioning was assessed using the Vineland Adaptive Behavior Scales, Second Edition—Survey Interview Form, a standardized parent interview that provides a general assessment of functioning across communication, daily living skills, and socialization domains.[22] The composite standard score was used as a measure of adaptive functioning.

RESULTS

Analytical Strategy

To investigate the main and interactive effects of the presence of any autism spectrum disorder (ASD)–associated copy number variants (CNVs) and self-reported infection or fever episode during pregnancy on ASD severity, we conducted a series of univariate 2-way analyses of variance to maximize sample size for each dependent variable. Due to the low frequency of each individual infection (Table 1), we did not investigate the effects of any single infection on autism severity and instead used a dichotomous variable to indicate whether the mother reported experiencing any infectious illness or febrile episode during her pregnancy. Finally, we separately examined the interactions between maternal infection or fever in pregnancy and presence of CNV on severity of symptoms on the Autism Diagnostic Interview-Revised (ADI-R), Social Responsiveness Scale (SRS), and Repetitive Behavior Scale-Revised (RBS-R) for individuals with deletions and duplications.

Several potential confounding factors were examined to determine whether they should be entered as covariates into statistical analyses. It is feasible that the earlier onset of ASD symptoms may relate to better recall of events on the medical history interview during pregnancy since events may have been more salient to parents at the time. However, the age that the first developmental abnormality manifested did not differ between probands exposed to infection during pregnancy versus those who were not ($t_{(1946)} = 0.39$, $p = .70$), suggesting that parental recall of events during pregnancy was not confounded with earlier symptom presentation. Additionally, proband age at the time of medical history interview did not differ between probands exposed to infection during pregnancy versus those who were not ($t_{(1989)} = 0.11$, $p = .92$); consequently, recall bias based on length of time since proband pregnancy is unlikely. Therefore, proband age and age of symptom presentation were not entered as covariates in analyses.

We also attempted to address the potential impact of several external factors on the presence of CNV status

Table 2. Clinical Phenotypes of Offspring Diagnosed with Autism Spectrum Disorder After Self-reported Infection or Fever During Pregnancy

	Autism Diagnostic Interview-Revised			
	Reciprocal Social Interaction, M (95% CI)	**Communication, M (95% CI)**	**Restrictive, Repetitive, Stereotyped Behaviors, M (95% CI)**	**Repetitive Behavior Scale, M (95% CI)**
No CNV				
No maternal infection	20.46 (20.16–20.75)	16.48 (16.25–16.72)	6.49 (6.36–6.62)	27.47 (26.59–28.36)
Maternal infection reported	19.62 (19.04–20.20)	16.35 (15.89–16.81)	6.76 (6.50–7.01)	27.84 (26.08–29.61)
CNV present				
No maternal infection	20.14 (19.14–21.14)	16.28 (15.47–17.08)	6.49 (6.05–6.93)	24.50 (21.46–27.54)
Maternal infection reported	22.66 (20.58–24.73)	18.69 (17.14–20.24)	8.03 (7.13–8.94)	34.21 (27.92–40.49)

	Autism Diagnostic Interview-Revised			
	Social Responsiveness Scale, M (95% CI)	**Verbal IQ, M (95% CI)**	**Nonverbal IQ, M (95% CI)**	**Vineland Adaptive Behavior Scales, M (95% CI)**
No CNV				
No maternal infection	79.77 (79.24–80.30)	77.13 (75.53–78.73)	84.56 (83.22–85.91)	72.97 (72.35–73.59)
Maternal infection reported	79.93 (78.87–80.99)	80.39 (77.20–83.57)	87.07 (84.39–89.76)	74.25 (73.02–75.48)
CNV present				
No maternal infection	77.82 (75.99–79.65)	76.46 (70.99–81.93)	80.02 (75.41–84.62)	71.61 (69.50–73.73)
Maternal infection reported	83.41 (79.65–87.18)	76.72 (65.41–88.04)	79.31 (69.78–88.84)	69.59 (65.22–73.96)

CNV, copy number variant.

and maternal infection. There were no differences between probands with/without CNVs and with/without maternal prenatal infections in maternal age at proband birth ($F_{(3,1955)} = 1.85$, $p = .14$), paternal age at proband birth ($F_{(3,1955)} = 1.24$, $p = .29$), maternal education ($F_{(3,1915)} = 0.38$, $p = .77$), and annual household income ($F_{(3,1829)} = 0.75$, $p = .52$). As a result, these demographic variables were not entered as controls into interaction analyses.

Interaction Between CNVs and Maternal Infection

As summarized in Table 2 and illustrated in Figure 1, we observed a statistically significant interactive effect of the presence of CNVs and maternal infection on all domains of the ADI-R, including Reciprocal Social Interaction (RSI; $F_{(1,1967)} = 7.56$, $p = .006$), Communication (COM; $F_{(1,1737)} = 7.54$, $p = .006$), and Restrictive, Repetitive, and Stereotyped Patterns of Behavior (RRSB; $F_{(1,1967)} = 5.74$, $p = .017$). Similarly, significant interactive effects were observed on impairment on the RBS-R ($F_{(1,1966)} = 6.36$, $p = .012$) and the SRS ($F_{(1,1958)} = 6.00$, $p = .014$). Individuals with ASD-associated CNVs and maternal infection had increased impairment across all measures relative to individuals with CNVs but no maternal infection, individuals with maternal infection but no CNVs, and individuals with neither risk factor. No significant interactive effects of the presence of a CNV and maternal infection or fever episode during pregnancy on cognitive or adaptive functioning were observed.

Deletions Versus Duplications

We observed a significant interaction between presence of a deletion of a region implicated in ASD and maternal self-reported infection or fever in pregnancy on ASD severity as measured by the ADI-R (RSI domain: $F_{(1,1900)} = 8.78$, $p = .003$; COM domain: $F_{(1,1676)} = 7.37$, $p = .007$; RRSB domain: $F_{(1,1900)} = 9.84$, $p = .002$). The interactive effect was observed when only individuals with duplications were compared with those without CNVs on the RBS-R: $F_{(1,1880)} = 6.88$, $p = .009$. No other significant interactive effects were observed (see Figure, Supplemental Digital Content 2, http://links.lww.com/JDBP/A69, which shows autism symptomatology and cognitive and adaptive functioning of children with ASD-associated CNVs and history of maternal infection or fever during pregnancy by type of copy number event, N = 1971, error bars = 95% confidence interval).

Main Effects of Presence of CNVs

There was a main effect of CNV presence on nonverbal IQ ($F_{(1,1968)} = 5.49$, $p = .019$) but not on verbal IQ ($F_{(1,1968)} = 0.225$, $p = .635$) and on adaptive

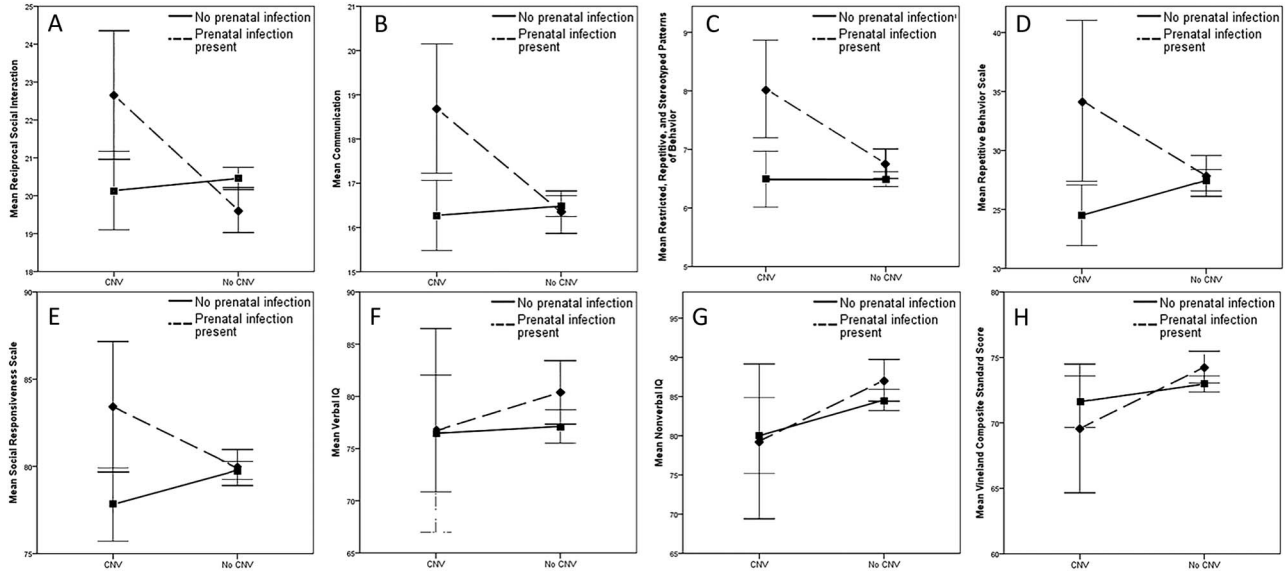

Figure 1. Autism symptomatology and cognitive and adaptive functioning of children with autism spectrum disorder–associated copy number variants (CNVs) and history of maternal infection or fever during pregnancy (N = 1971). Error bars = 95% confidence interval. As shown in graph C (p = .010), a main effect for presence of infections is demonstrated. As shown in graphs G (p = .019) and H (p = .049), main effects for presence of CNV are demonstrated. As shown in graphs A–E: (A) p = .006; (B) p = .006; (C) p = .017; (D) p = .012; (E) p = .014, significant interactions are observed. No significant interactions are observed in graphs F and H.

functioning ($F_{(1,1968)}$ = 3.87, p = .049), such that individuals with a CNV showed more impaired nonverbal IQ and poorer adaptive functioning. No main effects of the presence of a CNV were found on the ASD-related measures (ADI RSI domain: $F_{(1,1968)}$ = 0.442, p = .506; ADI COM domain: $F_{(1,1738)}$ = 0.746, p = .388; and ADI RRSB domain: $F_{(1,1968)}$ = 1.36, p = .243). No effects were found on social responsiveness by the SRS ($F_{(1,1959)}$ = 1.08, p = .299) nor on restrictive/repetitive behaviors by the RBS-R ($F_{(1,1967)}$ = 0.675, p = .411).

Copy number variants in our sample were evenly distributed concerning the inheritance pattern, with 23% (40 of 173) CNVs paternally inherited, 27% (47 of 173) maternally inherited, 25% de novo CNVs (43 of 173), and the rest (43 of 173) with unknown inheritance. A possible confounding interaction is that maternally inherited CNVs increase maternal susceptibility to infection as well as offspring ASD symptomatology. However, we found no difference in the prevalence of infections for individuals with maternally inherited CNVs (χ^2 = .526).

Main Effect of Maternal Infection

We saw a significant main effect of self-reported maternal infection or fever episode during pregnancy on the ADI-R in the RRSB domain ($F_{(1,1968)}$ = 6.67, p = .010), a trend toward significance in the RSI domain ($F_{(1,1968)}$ = 3.35, p = .068), and no effect in the COM domain ($F_{(1,1738)}$ = 0.074, p = .785). No effects were observed on the SRS ($F_{(1,1959)}$ = 0.935, p = .334) nor on the RBS-R ($F_{(1,1967)}$ = 1.20, p = .273). No main effects of maternal infection or fever during pregnancy on cognitive (Verbal IQ: $F_{(1,1968)}$ = 3.013, p = .083; Nonverbal IQ: $F_{(1,1968)}$ = 2.38, p = .123) or adaptive functioning ($F_{(1,1968)}$ = 2.36, p = .124) were observed.

DISCUSSION

We found strong evidence supporting the interaction between genetic susceptibility as defined by the presence of autism spectrum disorder (ASD)–associated copy number variants (CNVs) and environmental insults in the form of maternal infection or fever during pregnancy in the cause of increased severity of autism symptomatology in children with ASD. Individuals with a CNV and prenatal history of maternal infection/illness presented with greater autistic impairment, but showed no difference in cognitive or adaptive functioning, than children with either risk factor alone or neither risk factor. This pattern was reflected across all domains of ASD impairment and resulted in clinically relevant changes in behavioral outcomes. For instance, in the case of social responsiveness on the Social Responsiveness Scale (SRS), mean T scores bordered on the moderate/severe impairment cutoff for all groups except those individuals with both risk factors, whose mean score fell solidly in the severe category, using descriptive categories provided by the SRS (Table 2 and Fig. 1). The observed interactive effect between ASD-associated CNVs and maternal infection or fever in pregnancy on autism symptomatology as measured by the ADI-R was driven primarily by deletions.

In our large sample of well-characterized individuals with ASD, we extend the previous work that identifies genetic susceptibility[2–5] and environmental insults[6–8] as independent risk factors in ASD by illuminating the relationship between these risk factors and ASD

symptom presentation. Our findings support previous research on hot spots of recurrent CNVs associated with ASDs in which individuals with an identified CNV are more likely to demonstrate clinically related impairments.[3–5] Replicating results of previous work on CNV hot pots associated with cognitive and adaptive functioning, we found increased impairments in nonverbal IQ, but not verbal IQ, and poorer adaptive functioning associated with presence of any ASD-associated CNV.[5]

Our findings also support epidemiological and rodent model experiments that identify maternal immune activation (MIA) in pregnancy as associated with an increased risk of ASD. We observed significantly more impairment in repetitive and restrictive behaviors among children with ASD whose mothers reported prenatal infection or fever over 101°F during pregnancy with the proband. These findings mirror recent large epidemiological studies indicating approximately a 30% increase of risk in ASD when maternal infections result in hospitalization, suggesting that immune-mediated mechanisms play a role in the cause of ASD.[23] Our findings also reflect work on rodent models, which suggest that gestational infections, activating a maternal immune response, perturb fetal brain development mainly through interleukin (IL)-6 and result in offspring with the core autism characteristics.[9,10,24]

Most importantly, our finding of the significant interactive effect of ASD-associated CNVs and maternal infection or fever in pregnancy parallels recent rodent model work in characterizing the relationship between genetic susceptibility and environmental insult. Schwartzer et al[11] compared the effects of MIA on pups of mothers from an inbred strain predisposed to exhibit ASD-like behaviors to pups of a species typical strain and found greater behavioral impairment in pups with both genetic susceptibility and exposure to MIA than in pups with either risk factor alone, concluding that behavioral and immunological effects are strain dependent. Our similar finding that individuals genetically predisposed to ASD in the form of CNVs with exposure to maternal infection and fever had more severe behavioral phenotypes than those with the genetic risk factor or environmental insult alone further supports the hypothesis that the effect of maternal infection or immune activation may be dependent on genetic predisposition.

Interestingly, maternal infection alone and in interaction with ASD-associated CNVs affected ASD symptom severity but not cognitive or adaptive severity. Previous research suggests a link between elevated cytokine levels present in altered immune profiles and severity of behavioral outcomes, particularly with deficits in social interaction and communication.[25] Mouse models support the role of cytokines as putative in core behavioral features of autism: administration of IL-6 during pregnancy alone is enough to induce autism-like traits in the offspring,[9] and it has been proposed that cytokines affect behavior by altering levels of specific hormones, namely oxytocin and serotonin.[26] Furthermore, gene-ontology analysis of all mouse and human CNV genes has shown overrepresentation of immune system function and enrichment for neurodevelopment, specifically, for those within genomic hot spots.[27,28] Postmortem studies also found upregulated immune system genes in autism brains with environmental rather than genetic cause; these dysregulated immune-related genes seemed to also play a role in synaptic development and function.[29] A potential mechanism underlying the effects of maternal infection and fever on neural development that results in ASD-like behaviors is the major histocompatibility complex I–myocyte enhancer factor 2 pathway, which is altered in MIA offspring resulting in lower synapse density, and represents a common molecular pathway downstream of both genetic mutations and environmental factors that contribute to ASD.[30] Therefore, it is possible that CNVs that alter the immune system as well as pathways in neural synapse development, when combined with a severe activation of the maternal immune system early during gestation, lead to alteration in a common molecular pathway and result in the core behavioral features of ASD.

This study has several strengths: the Simons Simplex Collection is the largest collection of well-characterized children with ASD with complete phenotypic and genotypic information available to date, gathered as part of a collaborative multisite study. To our knowledge, this is the first study to investigate the interactive effects of genetic susceptibility and environmental insults on the degree of autistic impairment in humans. A consideration in the interpretation of this study is that we relied on parent self-report of infection during pregnancy. However, previous studies that have compared patient reports of illness in pregnancy against available medical records found fairly good agreement between the 2 sources, suggesting that maternal-reported data on infection is a valuable, albeit imperfect, source of medical history data.[7] In this study, we focused on CNVs in general, but future work should examine closer the role of other structural changes. In addition, we did not investigate the contributions of individual infections or specific recurrent CNVs on autism severity because of the rarity of specific events; a larger cohort study in future may be useful to elucidate the effects of particular infections on MIA and resultant autism impairment. Finally, all the children considered in this study have a diagnosis of ASD. As such, our conclusions extend previous epidemiological, genetic, and animal model work identifying risk factors for ASD by examining the specific contribution to ASD symptom presentation rather than autism etiology per se. Also, since ascertainment was limited to participants with ASD, our conclusions pertaining to cognitive and adaptive severity were restricted to some extent. Differential patterns of cognitive and adaptive severity in a sample ascertained on the diagnosis of intellectual disability would further elucidate the relationship between behavioral phenotypes

associated with maternal infection and CNVs. Future population-based studies are needed to further explore and characterize the relationship between these risk factors.

In conclusion, our results suggest that children with de novo or inherited ASD-associated CNVs may be more susceptible to environmental insults due to MIA during fetal development, as demonstrated by observed significantly stronger impairments across all domains of autism severity among our large sample of children with ASD. Although the discovery of CNVs associated with autism has advanced ASD understanding, further examination of the interactive factors that link genotype with the complex autistic presentation is still needed.

ACKNOWLEDGMENTS

The authors are grateful to all of the families at the participating Simons Simplex Collection (SSC) sites, as well as the principal investigators (A. Beaudet, R. Bernier, J. Constantino, E. Cook, E. Fombonne, D. Geschwind, R. Goin-Kochel, E. Hanson, D. Grice, A. Klin, D. Ledbetter, C. Lord, C. Martin, D. Martin, R. Maxim, J. Miles, O. Ousley, K. Pelphrey, B. Peterson, J. Piggot, C. Saulnier, M. State, W. Stone, J. Sutcliffe, C. Walsh, Z. Warren, and E. Wijsman). The authors appreciate obtaining access to phenotypic data on SFARI Base. Approved researchers can obtain the SSC population dataset described in this study by applying at https://base.sfari.org.

REFERENCES

1. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet.* 2008;9:341–355.
2. Sebat J, Lakshmi B, Malhotra D, et al. Strong association of de novo copy number mutations with autism. *Science*. 2007;316:445–449.
3. Luo R, Sanders SJ, Tian Y, et al. Genome-wide transcriptome profiling reveals the functional impact of rare de novo and recurrent CNVs in autism spectrum disorders. *Am J Hum Genet.* 2012;91:38–55.
4. Sanders SJ, Ercan-Sencicek AG, Hus V, et al. Multiple recurrent de novo CNVs, including duplications of the 7q11. 23 Williams syndrome region, are strongly associated with autism. *Neuron*. 2011;70:863–885.
5. Girirajan S, Dennis MY, Baker C, et al. Refinement and discovery of new hotspots of copy-number variation associated with autism spectrum disorder. *Am J Hum Genet.* 2013;92:221–237.
6. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry.* 2009;195:7–14.
7. Atladóttir HÓ, Henriksen TB, Schendel DE, et al. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics*. 2012;130:e1447–e1454.
8. Zerbo O, Iosif AM, Walker C, et al. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) study. *J Autism Dev Disord.* 2013;43:25–33.
9. Smith SE, Li J, Garbett K, et al. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci.* 2007;27:10695–10702.
10. Malkova NV, Yu CZ, Hsiao EY, et al. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain Behav Immun.* 2012;26:607–616.
11. Schwartzer JJ, Careaga M, Onore CE, et al. Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. *Transl Psychiatry.* 2013;3:e240.
12. Lord C, Risi S, Lambrecht L, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* 2000;30:205–223.
13. Lord C, Rutter M, Couteur A. Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord.* 1994;24:659–685.
14. American Psychiatric Association. *Diagnostic Criteria from DSM-IV-TR*. Washington, DC: American Psychiatric Association; 2000. xii, 370.
15. Fischbach GD, Lord C. The Simons simplex collection: a resource for identification of autism genetic risk factors. *Neuron*. 2010;68:192–195.
16. Lam KL, Aman M. The repetitive behavior scale-revised: independent validation in individuals with autism spectrum disorders. *J Autism Dev Disord.* 2007;37:855–866.
17. Mirenda P, Smith I, Vaillancourt T, et al. Validating the repetitive behavior scale-revised in young children with autism spectrum disorder. *J Autism Dev Disord.* 2010;40:1521–1530.
18. Constantino J, Davis S, Todd R, et al. Validation of a brief quantitative measure of autistic traits: comparison of the social responsiveness scale with the autism diagnostic interview-revised. *J Autism Dev Disord.* 2003;33:427–433.
19. Elliott CD. *Differential Ability Scales-II*. San Antonio, TX: Pearson; 2007.
20. Wechsler D. *Wechsler Intelligence Scale for Children - Third Edition (WISC-III)*. San Antonio, TX: The Psychological Corporation; 1991.
21. Mullen E. *Mullen Scales of Early Learning*. Circle Pines, MN: American Guidance Service. Inc(21); 1995.
22. Sparrow SS, Cicchetti DV. *The Vineland Adaptive Behavior Scales. Major Psychological Assessment Instruments*. Vol. 2. Needham Heights, MA: Allyn & Bacon; 1989:199–231.
23. Lee BK, Magnusson C, Gardner RM, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain Behav Immun.* 2014;44:100–105.
24. Hsiao EY, McBride SW, Chow J, et al. Modeling an autism risk factor in mice leads to permanent immune dysregulation. *Proc Natl Acad Sci U S A.* 2012;109:12776–12781.
25. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun.* 2012;26:383–392.
26. Miller VM, Zhu Y, Bucher C, et al. Gestational flu exposure induces changes in neurochemicals, affiliative hormones and brainstem inflammation, in addition to autism-like behaviors in mice. *Brain Behav Immun.* 2013;33:153–163.
27. Nguyen DQ, Webber C, Ponting CP. Bias of selection on human copy-number variants. *PLoS Genet.* 2006;2:e20.
28. Saxena V, Ramdas S, Ochoa CR, et al. Structural, genetic, and functional signatures of disordered neuro-immunological development in autism spectrum disorder. *PLoS One.* 2012;7:e48835.
29. Voineagu I, Wang X, Johnston P, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature*. 2011;474:380–384.
30. Elmer BM, Estes ML, Barrow SL, et al. MHCI Requires MEF2 Transcription factors to negatively regulate synapse density during development and in disease. *J Neurosci.* 2013;33:13791–13804.