Review

Biological Psychiatry

Genetic Epidemiology and Insights into Interactive Genetic and Environmental Effects in Autism Spectrum Disorders

Young Shin Kim and Bennett L. Leventhal

ABSTRACT

Understanding the pathogenesis of neurodevelopmental disorders has proven to be challenging. Using autism spectrum disorder (ASD) as a paradigmatic neurodevelopmental disorder, this article reviews the existing literature on the etiological substrates of ASD and explores how genetic epidemiology approaches including gene-environment interactions (G × E) can play a role in identifying factors associated with ASD etiology. New genetic and bioinformatics strategies have yielded important clues to ASD genetic substrates. The next steps for understanding ASD pathogenesis require significant effort to focus on how genes and environment interact with one another in typical development and its perturbations. Along with larger sample sizes, future study designs should include sample ascertainment that is epidemiologic and population-based to capture the entire ASD spectrum with both categorical and dimensional phenotypic characterization; environmental measurements with accuracy, validity, and biomarkers; statistical methods to address population stratification, multiple comparisons, and G × E of rare variants; animal models to test hypotheses; and new methods to broaden the capacity to search for G × E, including genome-wide and environment-wide association studies, precise estimation of heritability using dense genetic markers, and consideration of G × E both as the disease cause and a disease course modifier. Although examination of G × E appears to be a daunting task, tremendous recent progress in gene discovery has opened new horizons for advancing our understanding of the role of G × E in the pathogenesis of ASD and ultimately identifying the causes, treatments, and even preventive measures for ASD and other neurodevelopmental disorders.

Keywords: Autism spectrum disorders, Environment, Genes, Genetic epidemiology, Interactions, Neurodevelopmental disorders

http://dx.doi.org/10.1016/j.biopsych.2014.11.001

Pathogenesis of psychiatric disorders is complex, but gene discovery has provided insight into biological mechanisms underlying neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), intellectual disabilities, and schizophrenia. Gene discovery has led to only a modest understanding of ASD biological pathways; however, recent work with rare, de novo mutations is pointing the field in new directions, including converging actions of ASD-associated mutations and the rapidly evolving progress in the use of translational strategies (in animals and humans) to understand the effects of replicated ASD-associated mutations (1). Understanding roles of rare variants in ASD has highlighted the multifactorial etiology of NDDs characterized by pleiotropy (diverse phenotypes from identical genetic factors), genetic heterogeneity (different genes causing same phenotypes), and interactions: epistasis (between genes) and gene-environment interactions (G × E) (2–10). Evidence also suggests that environmental factors lead to diverse phenotypes, depending on the developmental timing of exposure (11–13). Research to disentangle this complexity requires strategies that specifically incorporate both genetic and environmental factors. This article provides 1) an overview of the role of genetic epidemiology in identifying both genetic and environmental factors and their joint roles in NDD etiology, 2) evidence regarding genetic and environmental influences on NDDs, and 3) research strategies to advance our understanding of NDD etiology. We use ASD as an exemplar of the broader group of NDDs.

GENETIC EPIDEMIOLOGY IN ASD

Genetic epidemiology uses disparate data from bioinformatics, population genetics, epidemiology, and molecular genetics to elucidate roles for genes and their interactions with environment in the occurrence of disease in populations (14). Genetic epidemiology 1) focuses on systematic sampling to enhance generalizability of research findings, 2) studies joint effects of genes and environment, and 3) incorporates disease biology into conceptual models (15). Twin, family, linkage, and association studies are among study designs that allow examination of genetic or environmental factors in diseases. With increasing evidence for NDD heterogeneity, genetic epidemiologic studies must attend to ASD phenotypic variability in both sampling and phenotype definition.

SEE COMMENTARY ON PAGE 3

 ISSN: 0006-3223

GENETICS

Twin studies with sample sizes of 11–67 monozygotic (MZ) and 9–210 dizygotic (DZ) twin pairs yield 47%–96% ASD concordance rates in MZ twins and 0–36% in DZ twins for autism and broader ASD phenotypes, suggesting strong heritability associated with ASD (16–21). Sibling relative risk (λ_s) (ratio of ASD prevalence among siblings of individuals with ASD to general population) ranges from 1.5–19.4 (22–26).

Initial genome-wide linkage studies were underpowered for detecting genes of small effect, leading to inconsistent findings across the samples and resistance to replication. Linkage analyses using larger samples, endophenotypes, and quantitative traits yielded positive findings in specific chromosomal regions: 2q, 5, 7q, 15q, and 16p (27,28).

Candidate gene association studies are used for ASD because they are more powerful than linkage, at a given locus, allowing detection of genes of weaker effect. Because of limited knowledge about ASD pathophysiology and gene functions, only a few genes, including *SLC6A4, GABR, RELN, NLGN, MET,* or *EN2,* have been examined with infrequent and inconsistent replications (27,29). Meta-analysis of 14 family-based association studies of *5HTT,* reported findings from each study as inconsistent, with main analyses showing no association (30).

In contrast, genome-wide association studies (GWAS) exploit strengths of association studies without guessing the identity of causal genes a priori (hypothesis-free approach); this leads to unbiased, comprehensive searches for susceptibility alleles (31). Although GWAS have yielded successes in medicine (e.g., age-related macular degeneration, obesity, hypertension, diabetes) (32–35), GWAS with samples >2000 failed to identify replicable common variants for ASD (36–39). Scarce replications in searches for ASD risk alleles result from 1) sample sizes insufficient to detect modest effect sizes; 2) poor control for population stratification in case-control studies; 3) overly permissive approaches in multiple comparison corrections, especially in early candidate gene studies; 4) varied ASD phenotype definitions; and 5) diverse samples primarily selected from nonrepresentative sources (40,41).

Significant increases in copy number variations (CNVs), which are submicroscopic variations in chromosomal structure, especially de novo CNVs, in simplex ASD families (i.e., only one affected individual) have been identified (42). Several investigators reported structural variations on the short arm of chromosome 16 associated with idiopathic ASD (42–45). This 16p11.2 CNV includes ~600 kilobases and ~29 genes, 22 of which are expressed in human fetal brain (46). Studies have demonstrated 16p11.2 deletions in ~.1%–.7% of ASD and 16p11.2 duplications in ~.1%–.5%, a rate 10 times greater than base rates for this CNV in the general population (7,47–49).

The 16p11.2 CNV is associated with the following phenotypes: intellectual disabilities, developmental delay, speech problems, schizophrenia, seizures, increased body weight or obesity, and increased head circumference (4–9,48–54). Similar phenomena have been reported for a large number of ASD-associated CNVs, including CNVs on 1q21.2, 3q29, 7q11.23, 7q36.3, 15q11.2, 15q13.3, 16p13.11, 17p12, 17q12, and 22q11.21 (3).

Excess de novo single nucleotide variant burden has been observed only for loss of function (i.e., nonsense, canonical splice site, and frameshift mutations) (55–60). Multiple de novo single nucleotide variants at the same locus, compared with the null distribution in control subjects, allowed identification of genome-wide significant loci, including loss of function mutations in *SCN2A, CHD8, DYRK1A, GRIN2B, KATNAL2, POGZ, CUL3,* and *TBR1* (55–58,61). Similar to CNVs, some single nucleotide variants initially associated with single disorders are now associated with other disorders (e.g., *SCN2A* in ASD and epilepsy) (55,57,62).

More recent studies confirm ASD-related genetic heterogeneity found in earlier twin, family, and linkage studies. The ASD-related genes seem to converge on a few pathophysiologic pathways related to synaptic function and plasticity, GTPase and Ras signaling, and neurogenesis (45,63–67). Phenotype pleiotropy suggests interaction with additional genetic and nongenetic factors, acting at various developmental time points and resulting in divergent phenotype manifestations of a single genetic variant (68).

ENVIRONMENTAL FACTORS

Twin studies provide strong evidence equally for genetics and environmental factors in ASD risk. High levels of heritability (phenotypic variance owing to genetic factors), in the range of ~90%, were reported in early twin studies; a more recent twin study found larger environmental influences on ASD risk—37% heritability and 55% shared environmental liability (20,69,70). These findings have been replicated in a large independent population-based Swedish National Registry study of 2,049,973 siblings including DZ and MZ twins, yielding 50% heritability and 50% nonshared environmental influence for ASD (26). Diagnostic disparities in some MZ twin pairs also suggest that environmental factors contribute to both liability for and expression of autism-related traits (71).

Progressively higher ASD prevalence estimates (.07%–2.6%) suggest that most of the increase is attributable to greater public awareness, better case ascertainment, broadening of ASD diagnostic construct, and diagnostic substitution (72–74). If increasing ASD prevalence is even partly caused by increasing incidence, environmental factors or their interactions with as-yet-unknown genetic vulnerability may represent other ASD risk mechanisms.

Nutrients, smoking, alcohol, medications, and pesticides are the most commonly examined exposures during pregnancy because of their known neurotoxicity or specific adverse or protective impacts on developing brains (75). Epigenetics (long-term or heritable changes in function of a locus or chromosome without alteration of underlying DNA) may represent one pathway for G×E (76). There is an association of ASD with fragile X, Rett, and Angelman syndromes, each of which involves epigenetic mechanisms (77–79). Associations have been also reported between ASD and parent-of-origin syndromes (e.g., 15q11.3, Turner syndrome) (10,75,80). Two recent studies report differences in DNA methylation profiles between individuals with and without ASD; one group studied 20 postmortem brains of individuals with ASD and 21 of control subjects, finding differentially

methylated regions in or near genes implicated in cell signaling, synaptic function, plasticity, imprinting, and metabolism (80). Analyses of 6 discordant and 44 concordant MZ twins found numerous differentially methylated regions associated with ASD in within-twin and between-group analyses. Significant correlations between DNA methylation and quantitative autistic traits were also found (81); these findings should be interpreted with caution because of methodologicl limitations, including small nonrepresentative samples, inadequate case-control sample comparability, multiple comparisons, and cross-sectional design that cannot discern causal relationship. The substrate for epigenetic dysregulation is unknown; however, environmental factors are associated with epigenetic changes, including correlations between folate levels and global DNA methylation (75). These correlations provide evidence that environmental factors may contribute to ASD etiology through epigenetic processes (82,83).

Studies report associations between ASD and prenatal toxic exposure, ranging from thalidomide to maternal, intrapartum rubella (84–88), suggesting that environmental exposures during critical periods contribute to ASD susceptibility. How this occurs, the timing, and direct effects causing ASD remain unknown.

Two meta-analyses of 24 studies examined associations between ASD and perinatal complications (89,90), finding modest increases in ASD risk. Odds ratios (OR) from 1.4–1.8 were associated with abnormal fetal presentation, umbilical cord complications, fetal distress, multiple birth, maternal hemorrhage, summer birth, small size for gestational age, congenital malformation, meconium aspiration, ABO or Rh incompatibility, and hyperbilirubinemia, with the largest ORs for birth injury (OR = 4.9), low birth weight (<1500 g) (OR = 3.0), and neonatal anemia (OR = 7.87). Although disaggregating perinatal complications from ASD is challenging because susceptibility might have led to perinatal complications and ASD, two hypotheses exist:

- Hypoxia: Obstetric complications related to hypoxia pose significant risk in meta-analyses (90). This hypothesis is supported by a recent twin study indicating that respiratory distress and other markers of hypoxia increased ASD risk (91). Relationships between brain hypoxia and social behavior deficits warrant further attention.
- Immunologic: Studies link maternal infections during pregnancy to ASD in offspring (89,90). Although not well established, this association may be a direct effect of the infectious agent or the resulting activation of the maternal immune system leading to increased ASD risk (92–95). Approximately 10% of mothers of children with ASD harbor antibrain antibodies that bind to fetal brain (96,97); antibrain antibody assays were conducted only in mothers of case children and not in mothers of control children. When these antibodies were administered to gestating mice and monkeys, the offspring exhibited abnormal behaviors, including social deficits (98–101). These studies suggest that maternal antibodies can alter fetal development resulting in ASD-like behaviors.

Immunologic hypotheses are appealing when combined with transcriptomic analyses of postmortem brain of individuals with autism (102). Compared with individuals without ASD, brains of individuals with ASD demonstrated down-regulation of 209 genes, enriched for gene categories related to synaptic function, whereas 235 genes implicated in immune and inflammatory response were upregulated. The former group was significantly enriched for association signals in GWAS, whereas the latter group was not. These studies suggest that relevant immunologic changes are likely caused by environmental factors; however, the roles of rare variants resulting in observed findings cannot be ruled out.

Two studies report associations between ASD and folate exposure. Each study reported protective effects of intake at different times—first trimester versus preconception—suggesting that there may be multiple points during fetal development when folate is protective (103,104).

The roles of advanced maternal and paternal age at pregnancy on ASD risk in offspring have been consistently reported (105,106). Suggested mechanisms include increased rates of de novo mutation, epigenetic dysfunction, and cumulative exposure to environmental toxins (60,105,107).

Studies examining relationships between maternal smoking or drinking during pregnancy and ASD risk are inconsistent and inconclusive. No studies examined the impact of paternal exposure on ASD risks (108). Exposures to antiepileptic medications and antidepressants, before and during pregnancy, suggest increased risk for ASD; however, exposure timing differed in each study, making sound conclusions challenging (108). Studies of pesticide exposure are still too few to draw conclusions.

Inconsistencies in findings and methodologic differences in ASD environmental studies make it challenging to reach solid conclusions; inconsistent results are not surprising given significant methodologic differences in work to date. Phenotype measurement differences occur because 1) quality of ASD diagnoses varies greatly; 2) comorbidity, especially intellectual disabilities, is not considered, making it difficult to disaggregate risk factors for intellectual disabilities or other comorbidity from those for ASD; and 3 case ascertainment from administrative datasets increases likelihood of ascertainment bias because of factors associated with accessibility or eligibility for inclusion. Administrative datasets have great diversity because inclusion criteria are administratively but not clinically salient and may change over time. Measurement differences in these studies include 1) varying methods to measure perinatal risks; 2) timing and dose of exposure not consistently measured, making it difficult to align exposure with specific developmental processes and to compare exposures across studies; 3) validity of exposure data jeopardized by retrospective recall; and 4) ecologic fallacy present when group level exposures are obtained.

G × E

Genes and environment rarely act alone to create ASD or other NDDs (7). Despite many studies exploring roles of genes or environmental factors in ASD, few examine G × E. Because development is a dynamic process reflecting a constant interplay between genes and environment, these interactions occur constantly. Specific perturbations in this process likely play a role in ASD etiology; ignoring these interactions may

obscure independent genetic or environmental effects, leading to false-negative and inconsistent findings (109). Both human and animal studies suggest that G×E plays a role in ASD pathogenesis (10).

When considering G×E, it is important to distinguish 1) statistical versus biological interaction, 2) additive versus multiplicative interaction, and 3) gene-environmental correlation (rGE) versus G×E. Statistical interaction of multiple factors is the coefficient of the product term of the risk factors. Although convenient for identifying interactions, it ignores biological plausibility of interaction mechanisms and depends on statistical models (110). Statistical interactions can be artifactual by data anomalies, including data distribution problems, making a careful examination of data characteristics essential when statistical interactions are detected (111). Biological interaction refers to multiple biological factors acting together to increase or decrease disease risk or the pathophysiologic substrate of disorders (112). An example is phenylketonuria, caused by genetic mutation. Having the mutation is not sufficient; environmental phenylalanine in addition to the genetic anomaly is required to create the disease phenotype. Disruption of that interaction (phenylalanine-restricted diet) is sufficient intervention to treat the condition.

Interactions can be detected as multiplicative or additive. Multiplicative interactions exist when the relative risk of having multiple factors does not equal the product of the relative risks associated with each factor separately (113). Logistic regression implicitly uses multiplicative scales (112). Additive interactions exist when the excess risk attributable to multiple factors does not equal the sum of excess risk caused by each factor separately (113). Despite the analytic convenience of multiplicative interactions, use of additive interactions is advocated when examining biological interactions because of its public health implications (114). Because detection of interactions is depends on the model used, it is possible to have one significant additive or multiplicative interaction but not the other (111). For example, bladder cancer risk (OR) in individuals with the chr1p13.3 deletion is 1.70 (95% confidence interval, 1.38–2.09), in individuals with smoker status is 3.30 (95% confidence interval, 2.71–4.03), and in individuals with the joint effect is 4.69 (95% confidence interval, 3.86–5.69); the null risks under additive and multiplicative interaction models are 4.00 and 5.61, respectively, making G×E significant only in the additive model (115).

Genetic difference in vulnerability to particular environmental factors that contribute to a phenotype defines G×E. The genetic difference that causes differential exposure to particular environment factors defines rGE; rGE may be heritable, even if consequences of exposure are not (116,117). For example, individuals with antisocial behavior are more likely to engage in risk-taking behaviors such as drinking and driving (118); however, increased risk for automobile accidents associated with drinking and driving is an rGE and not genetic. To avoid mistaking rGE as genetic effects or G×E, investigators must be aware that even small rGE may cause type I errors in G×E studies with case-only or case-control designs (117).

Several research designs are useful for G×E studies. When shared or nonshared environment data are available, twin studies can provide inferences about specific G×E (119). Discordant MZ twin, adoption, and half-sibling studies can identify environmental factors and G×E (120). Nested case-control studies offer advantages by minimizing selection and recall biases; however, they are not optimal for rare diseases, and selection bias can occur from low participation rates (119). Although case-only designs are efficient for examining G×E, they are based on no rGE and rare disease assumptions; even minor violations of these assumptions cause significant bias (121,122). In discordant sibling studies, unaffected siblings had fewer perinatal complications than ASD probands but more than controls, suggesting that individuals with ASD react differently to the same environmental stimuli and have less tolerance to perinatal environment compared with siblings (10,123).

Inconsistent findings regarding parental smoking and ASD risks may be explained by G×E. One study comparing stillbirths of smoking and nonsmoking mothers found *EN2* strongly expressed in arcuate nucleus neurons in fetuses of nonsmokers but not in 11 of 12 fetuses of smoking mothers; however, small sample size and multiple comparisons hamper the interpretations of the results (124). Intrauterine smoke exposure also damages serotonin projections in cortex and striatum, producing sex-selective changes in 5-hydroxytryptamine 1A and 5-hydroxytryptamine 2 receptor expression and inducing adenyl cyclase causing sensitization of heterologous inputs in this signaling pathway (125,126). *EN2* and *SLC6A4* were associated with ASD in candidate gene studies but not in GWAS. The GWAS replication failures might stem partially from failure to consider interactions between environmental events (in utero nicotine exposure) and genetic vulnerabilities (*EN2* or *SLC6A4* variants) in increased ASD risk. These G×E hypotheses require further examination.

Animal studies support G×E in ASD pathogenesis. Pletnikov *et al.* (127) reported differences in brain pathology, behavior, neurochemistry, and drug response in rats exposed to Borna disease virus, a teratogen causing neurodevelopmental damage and behavioral deficits analogous to ASD. Other animal models, ranging from *Caenorhabditis elegans* to mice, also demonstrate that genetic defects in synaptic function alter sensitivity to environmental factors, suggesting plausible G×E mechanisms (10).

Using a case-control design, one group reported three G×E from the same study population. In the study of 429 children with ASD and 278 typical children, maternal *MTHFR 677TT* (rs1801133), *CBS* rs234715 GT+TT, and child *COMT 472AA* (rs4680) genotypes conferred greater ASD risk when the mother did not take vitamins periconceptionally (128). However, observed G×E lost significance after multiple comparison corrections. Also, population stratification was not controlled for when investigators reported differences in racial composition between cases and controls. In the second study of 429 children with ASD, 130 children with developmental disorders, and 278 typically developing children, the strongest protective effects of maternal folate intake during the first month of pregnancy were reported in mothers and children with the *MTHFR* 677C>T variant (103). Multiple comparisons were not addressed, and residual population stratification, resulting from crude racial categories and retrospective exposure data collection, are sources of type I error.

The third study reported interactions between high pollution and nitrogen dioxide and the *MET CC* genotype (rs1858830) in 251 cases and 156 control subjects. Air pollution was determined using public air quality data for the area where individuals reported residence at birth (129). In addition to uncorrected multiple comparisons and population stratification, pollution exposure was measured at a group level leading to concern about ecological fallacy and type I error. All three studies used a candidate gene approach prone to false-positive findings (130). None of these G×E findings have been independently replicated.

CHALLENGES IN G×E RESEARCH AND FUTURE SUGGESTIONS

Studies involving G×E are power-intensive. Even testing for a single G×E specified a priori, the exponential growth in the number of comparisons requires large samples (131). For example, in an unmatched case-control study with a log-additive inheritance model, sample sizes required to examine a G×E effect size of OR 1.5 and 2.0 with 80% power and two-tail *p* value .05 (without multiple comparison corrections) are 31,084 and 9550, respectively, when prevalence of a disease, environmental risk, and a risk allele are 1%, 5%, and 5% and main effects of environmental and genetic risks are OR 1.2 and 1.2 (132). Researchers attempt to overcome this challenge in two ways: increasing sample size by establishing large consortia that share data and meta-analyses. Several large-scale, population-based genetic epidemiologic ASD studies are underway, including Norwegian Autism Birth Cohort Study and Mother and Child Cohort Study, Danish Birth Cohort Study, Finnish Birth Cohort Study, and Swedish Registry–based studies as well as U.S.-based Childhood Autism Risks from Genetics and Environment Study and Early Autism Risk Longitudinal Investigation Study (92,104,133–137). All these studies except Childhood Autism Risks from Genetics and Environment use prospective cohort designs; European studies use population-based representative samples. Biological specimens are collected during pregnancy with repeated phenotype and exposure measurements during follow-up. When combined with attempts to harmonize the exposure and phenotype measurements across studies, power to detect G×E is significantly increased.

There are not enough methodologically sound G×E ASD studies to conduct meta-analyses. Investigators wishing to conduct valid meta-analyses need ASD G×E studies with ample size, appropriately ascertained samples, detailed phenotypes, sound environmental measurement, state-of-the-art sequencing data, and strong statistical analyses. Investigators and editors must publish both positive and negative findings to minimize publication bias (131).

Research identifying how environmental factors affect ASD pathogenesis has often been characterized by relatively low levels of accuracy and reliability in the measurement of environmental exposures, leading to both type I and II errors in G×E studies. Environmental measures in ASD G×E studies were based on questionnaire or ecological measures rather than necessary measurement at the individual level. In the cited air pollution G×E study, linking group-level exposures of air pollution to individual-level exposure is difficult because it depends on specific information on exposure duration and individual biological factors, including inhalation or absorption of pollutants, activities, ages, and preexisting health conditions (138). There is no information about how maternal air pollutant exposure is correlated to fetal exposure in humans. Solving these problems is critical for identifying biomarkers of individual exposures (parental and child) and understanding specific environmental risk and its influence on ASD pathogenesis. Forthcoming technologies, such as geographic information systems and biological monitoring and sensing, enable more precise measurement of environmental exposure at individual levels (138). Meanwhile, researchers must use established methods to reduce measurement error, including validity and reliability studies for questionnaires and ecological data (139).

Systematically ascertained population-based samples representing the entire spectrum of the ASD phenotype are optimal for G×E studies. For cost and convenience, clinical or administrative samples are used more commonly even though they are more vulnerable to selection bias in terms of case severity, comorbidity, and factors associated with access to services (140,141). For example, two studies revealed that sex prevalence discrepancy decreased from 4–5:1 to 2–2.5:1 in epidemiologically ascertained children with ASD (26,73). Similarly, the proportion of children with ASD and intellectual disabilities decreases significantly as community ascertainment is more complete (73,142).

Heterogeneity of ASD phenotype poses challenges in etiological research, including G×E studies. Dimensional phenotyping using tools already available (e.g., Social Responsiveness Scale) is less vulnerable to phenotype heterogeneity and can complement traditional diagnostic approaches (143,144).

Methodologic advances can address preexisting challenges in G×E studies. Experimental models allow G×E testing in animal and cellular systems allowing subsequent population-based G×E studies with more specificity (145). Advances in technology will allow examination of genetic and environmental factors for ASD risks without a priori hypotheses about candidate genetic and environmental factors (146). There is already successful gene discovery using agnostic approaches through GWAS (147). Similarly, proof-of-principle environment-wide association studies are being examined (146). When methods are well established, environment-wide association studies combined with GWAS will provide opportunities for identifying novel G×E mechanisms (148).

CONCLUSIONS

New genetic and bioinformatics strategies have yielded important clues to ASD genetic architecture. Recently, a large, epidemiologically ascertained ASD sample estimated liabilities of 2.6% from rare de novo mutation, 3% from rare inherited variants, and 49% from common inherited variants (149). Understanding ASD pathogenesis will require more sophistication. Significant effort must focus on how genes and environment interact with one another in typical development and its perturbations, including perturbations that yield ASD. Larger sample sizes are necessary, but they are not sufficient to address complex questions. Study designs should include

the following: 1) sample ascertainment that is epidemiologic and population-based to capture the entire ASD spectrum; 2) phenotypic characterization that is both categorical and dimensional; 3) environmental measurement with accuracy, validity, and biomarkers; 4) statistical methods that address challenges such as population stratification, multiple comparisons, and G×E with rare genetic variants (e.g., rare variant burden analyses stratified by environmental exposure status in cases); 5) animal models to test hypotheses before and after conducting studies in human subjects; and 6) new methods including GWAS and environment-wide association studies to broaden the capacity to search for G×E, precise partitioning of heritability with the use of dense genetic markers from GWAS to refine the search for G×E, and consideration of G×E not only as the cause of disease but also as a disease course and outcome modifier (150).

We have the tools to study and understand G×E with recently identified genes; application of methodologically rigorous studies has major potential for discovering new ASD etiological substrates. Although examination of G×E appears to be a daunting task, tremendous recent progress in gene discovery opens new horizons for advancing our understanding of the role of G×E in the pathogenesis of ASD and ultimately identifying the causes, treatments, and even preventive measures for ASD and other NDDs.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the National Institutes of Health Grant No. R01 ES021462.

BLL has consulted for Janssen and has received a research grant from Roche. YSK reports no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Department of Psychiatry, University of California, San Francisco, San Francisco, California; and Department of Psychiatry, Yonsei University College of Medicine, Seoul, South Korea.

Address correspondence to Young Shin Kim, M.D., M.S., M.P.H., Ph.D., Department of Psychiatry, University of California, San Francisco, 401 Parnassus Avenue, San Francisco, CA 94143-0984; E-mail: youngshin.kim@ucsf.edu.

Received Feb 3, 2014; revised Oct 31, 2014; accepted Nov 2, 2014.

REFERENCES

1. Willsey AJ, Sanders SJ, Li M, Dong S, Tebbenkamp AT, Muhle RA, *et al*. (2013): Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. Cell 155: 997–1007.
2. Kim YS, State MW (2014): Recent challenges to the psychiatric diagnostic nosology: A focus on the genetics and genomics of neurodevelopmental disorders. Int J Epidemiol 43:465–475.
3. Malhotra D, Sebat J (2012): CNVs: Harbingers of a rare variant revolution in psychiatric genetics. Cell 148:1223–1241.
4. Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, van Haeringen A, Fransen van de Putte DE, Anderlid BM, *et al*. (2009): Extending the phenotype of recurrent rearrangements of 16p11.2: Deletions in mentally retarded patients without autism and in normal individuals. Eur J Med Genet 52:77–87.
5. Mefford HC, Cooper GM, Zerr T, Smith JD, Baker C, Shafer N, *et al*. (2009): A method for rapid, targeted CNV genotyping identifies rare variants associated with neurocognitive disease. Genome Res 19: 1579–1585.
6. Zufferey F, Sherr EH, Beckmann ND, Hanson E, Maillard AM, Hippolyte L, *et al*. (2012): A 600 kb deletion syndrome at 16p11.2 leads to energy imbalance and neuropsychiatric disorders. J Med Genet 49:660–668.
7. Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, *et al*. (2011): Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. Neuron 70:863–885.
8. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, *et al*. (2009): Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet 41:1223–1227.
9. Ahn K, Gotay N, Andersen TM, Anvari AA, Gochman P, Lee Y, *et al*. (2014): High rate of disease-related copy number variations in childhood onset schizophrenia. Mol Psychiatry 19:568–572.
10. Chaste P, Leboyer M (2012): Autism risk factors: Genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 14:281–292.
11. Knudsen AK, Skogen JC, Ystrom E, Sivertsen B, Tell GS, Torgersen L (2014): Maternal pre-pregnancy risk drinking and toddler behavior problems: The Norwegian Mother and Child Cohort Study. Eur Child Adolesc Psychiatry 23:901–911.
12. Larkby CA, Goldschmidt L, Hanusa BH, Day NL (2011): Prenatal alcohol exposure is associated with conduct disorder in adolescence: Findings from a birth cohort. J Am Acad Child Adolesc Psychiatry 50:262–271.
13. May PA, Blankenship J, Marais AS, Gossage JP, Kalberg WO, Joubert B, *et al*. (2013): Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): Quantity, frequency, and timing of drinking. Drug Alcohol Depend 133:502–512.
14. Khoury MJ, Beaty TH, Cohen BH (1993): 3rd ed.In: Fundamentals of Genetic Epidemiology. New York: Oxford University Press.
15. Thomas DC (2000): Genetic epidemiology with a capital "E." Genet Epidemiol 19:289–300.
16. Rosenberg RE, Law JK, Yenokyan G, McGready J, Kaufmann WE, Law PA (2009): Characteristics and concordance of autism spectrum disorders among 277 twin pairs. Arch Pediatr Adolesc Med 163: 907–914.
17. Folstein S, Rutter M (1977): Genetic influences and infantile autism. Nature 265:726–728.
18. Lichtenstein P, Carlstrom E, Rastam M, Gillberg C, Anckarsater H (2010): The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry 167: 1357–1363.
19. Bohm HV, Stewart MG (2009): Brief report: On the concordance percentages for autistic spectrum disorder of twins. J Autism Dev Disord 39:806–808.
20. Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, *et al*. (2011): Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry 68:1095–1102.
21. Nordenbaek C, Jorgensen M, Kyvik KO, Bilenberg N (2014): A Danish population-based twin study on autism spectrum disorders. Eur Child Adolesc Psychiatry 23:35–43.
22. Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, Zwaigenbaum L, *et al*. (2011): Recurrence risk for autism spectrum disorders: A Baby Siblings Research Consortium study. Pediatrics 128: e488–e495.
23. Georgiades S, Szatmari P, Zwaigenbaum L, Bryson S, Brian J, Roberts W, *et al*. (2013): A prospective study of autistic-like traits in unaffected siblings of probands with autism spectrum disorder. JAMA Psychiatry 70:42–48.
24. Gronborg TK, Schendel DE, Parner ET (2013): Recurrence of autism spectrum disorders in full- and half-siblings and trends over time: A population-based cohort study. JAMA Pediatr 167:947–953.
25. Constantino JN, Todorov A, Hilton C, Law P, Zhang Y, Molloy E, *et al*. (2013): Autism recurrence in half siblings: Strong support for genetic mechanisms of transmission in ASD. Mol Psychiatry 18: 137–138.

26. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A (2014): The familial risk of autism. JAMA 311:1770–1777.
27. Veenstra-VanderWeele J, Cook EH Jr (2004): Molecular genetics of autism spectrum disorder. Mol Psychiatry 9:819–832.
28. Li X, Zou H, Brown WT (2012): Genes associated with autism spectrum disorder. Brain Res Bull 88:543–552.
29. Veenstra-VanderWeele J, Christina SL, Cook EHJ (2004): Autism as a paragmatic complex genetic disorder. Annu Rev Genomics Hum Genet 5:379–405.
30. Huang CH, Santangelo SL (2008): Autism and serotonin transporter gene polymorphisms: A systematic review and meta-analysis. Am J Med Genet B Neuropsychiatr Genet 147B:903–913.
31. Hirschhorn JN, Daly MJ (2005): Genome-wide association studies for common diseases and complex traits. Nat Rev Genet 6:95–108.
32. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, *et al*. (2005): Complement factor H polymorphism in age-related macular degeneration. Science 308:385–389.
33. Berndt SI, Gustafsson S, Magi R, Ganna A, Wheeler E, Feitosa MF, *et al*. (2013): Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. Nat Genet 45:501–512.
34. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, *et al*. (2007): Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316:1331–1336.
35. Wain LV, Verwoert GC, O'Reilly PF, Shi G, Johnson T, Johnson AD, *et al*. (2011): Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet 43: 1005–1011.
36. Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, *et al*. (2009): Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature 459:528–533.
37. Weiss LA, Arking DE, Daly MJ, Chakravarti A (2009): A genome-wide linkage and association scan reveals novel loci for autism. Nature 461:802–808.
38. Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, *et al*. (2012): Individual common variants exert weak effects on the risk for autism spectrum disorderspi. Hum Mol Genet 21:4781–4792.
39. Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, *et al*. (2010): A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet 19:4072–4082.
40. Rutter M, Silberg J, O'Connor T, Simonoff E (1999): Genetics and child psychiatry: I. Advances in quantitative and molecular genetics. J Child Psychol Psychiatry 40:3–18.
41. Moldin SO (1997): The maddening hunt for madness genes. Nat Genet 17:127–129.
42. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, *et al*. (2007): Strong association of de novo copy number mutations with autism. Science 316:445–449.
43. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, *et al*. (2008): Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 358:667–675.
44. Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, *et al*. (2008): Recurrent 16p11.2 microdeletions in autism. Hum Mol Genet 17:628–638.
45. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, *et al*. (2008): Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488.
46. Simons VIP Consortium (2012): Simons Variation in Individuals Project (Simons VIP): A genetics-first approach to studying autism spectrum and related neurodevelopmental disorders. Neuron 73:1063–1067.
47. Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB, *et al*. (2010): Clinical genetic testing for patients with autism spectrum disorders. Pediatrics 125:e727–e735.
48. Jacquemont S, Reymond A, Zufferey F, Harewood L, Walters RG, Kutalik Z, *et al*. (2011): Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. Nature 478: 97–102.
49. Guha S, Rees E, Darvasi A, Ivanov D, Ikeda M, Bergen SE, *et al*. (2013): Implication of a rare deletion at distal 16p11.2 in schizophrenia. JAMA Psychiatry 70:253–260.
50. Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, *et al*. (2010): Large, rare chromosomal deletions associated with severe early-onset obesity. Nature 463:666–670.
51. Walters RG, Coin LJ, Ruokonen A, de Smith AJ, El-Sayed Moustafa JS, Jacquemont S, *et al*. (2013): Rare genomic structural variants in complex disease: Lessons from the replication of associations with obesity. PLoS One 8:e58048.
52. Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, *et al*. (2010): A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature 463:671–675.
53. Steinberg S, de Jong S, Mattheisen M, Costas J, Demontis D, Jamain S, *et al*. (2014): Common variant at 16p11.2 conferring risk of psychosis. Mol Psychiatry 19:774–783.
54. Grozeva D, Conrad DF, Barnes CP, Hurles M, Owen MJ, O'Donovan MC, *et al*. (2012): Independent estimation of the frequency of rare CNVs in the UK population confirms their role in schizophrenia. Schizophr Res 135:1–7.
55. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, *et al*. (2012): De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485: 237–241.
56. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, *et al*. (2012): Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485:246–250.
57. Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, *et al*. (2012): Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485:242–245.
58. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, *et al*. (2012): De novo gene disruptions in children on the autistic spectrum. Neuron 74:285–299.
59. Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, *et al*. (2012): Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. Cell 151:1431–1442.
60. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, *et al*. (2012): Rate of de novo mutations and the importance of father's age to disease risk. Nature 488:471–475.
61. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, *et al*. (2012): Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 338: 1619–1622.
62. Epi4K Consortium; Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, *et al*. (2013): De novo mutations in epileptic encephalopathies. Nature 501:217–221.
63. Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, *et al*. (2010): Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42: 489–491.
64. Pinto D, Pagnamenta AT, Klei L, Anney R, Merico D, Regan R, *et al*. (2010): Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466:368–372.
65. Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D (2011): Rare de novo variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. Neuron 70:898–907.
66. State MW, Sestan N (2012): Neuroscience. The emerging biology of autism spectrum disorders. Science 337:1301–1303.
67. Ben-David E, Shifman S (2012): Networks of neuronal genes affected by common and rare variants in autism spectrum disorders. PLoS Genet 8:e1002556.
68. Robinson MR, Wray NR, Visscher PM (2014): Explaining additional genetic variation in complex traits. Trends Genet 30:124–132.
69. Folstein S, Rutter M (1977): Infantile autism: A genetic study of 21 twin pairs. J Child Psychol Psychiatry 18:297–321.
70. Ritvo ER, Freeman BJ, Mason-Brothers A, Mo A, Ritvo AM (1985): Concordance for the syndrome of autism in 40 pairs of afflicted twins. Am J Psychiatry 142:74–77.

71. Muhle R, Trentacoste SV, Rapin I (2004): The genetics of autism. Pediatrics 113:e472–e486.
72. Gillberg C (1984): Infantile autism and other childhood psychoses in a Swedish urban region. Epidemiological aspects. J Child Psychol Psychiatry 25:35–43.
73. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, *et al*. (2011): Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry 168:904–912.
74. Fombonne E (2009): Epidemiology of pervasive developmental disorders. Pediatr Res 65:591–598.
75. LaSalle J (2013): Epigenomic strategies at the interface of genetic and environmental risk factors for autism. J Hum Genet 58:396–401.
76. Goldberg AD, Allis CD, Bernstein E (2007): Epigenetics: A landscape takes shape. Cell 128:635–638.
77. Belmonte MK, Bourgeron T (2006): Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 9: 1221–1225.
78. Percy AK (2011): Rett syndrome: exploring the autism link. Arch Neurol 68:985–989.
79. Bonati MT, Russo S, Finelli P, Valsecchi MR, Cogliati F, Cavalleri F, *et al*. (2007): Evaluation of autism traits in Angelman syndrome: A resource to unfold autism genes. Neurogenetics 8:169–178.
80. Ladd-Acosta C, Hansen KD, Briem E, Fallin MD, Kaufmann WE, Feinberg AP (2014): Common DNA methylation alterations in multiple brain regions in autism. Mol Psychiatry 19:862–871.
81. Wong CCY, Meabum EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, *et al*. (2014): Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. Mol Psychiatry 19:495–503.
82. Shi M, Christensen K, Weinberg CR, Romitti P, Bathum L, Lozada A, *et al*. (2007): Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. Am J Hum Genet 80: 76–90.
83. Kohlmeier M, Dda Costa KA, Fischer LM, Zeisel SH (2005): Genetic variation of folate-mediated one-carbon transfer pathway predicts susceptibility to choline deficiency in humans. Proc Natl Acad Sci U S A 102:16025–16030.
84. Chess S (1971): Autism in children with congenital rubella. J Autism Child Schizophr 1:33–47.
85. Moore SJ, Turnpenny P, Quinn A, Glover S, Lloyd DJ, Montgomery T, *et al*. (2000): A clinical study of 57 children with fetal anticonvulsant syndromes. J Med Genet 37:489–497.
86. Bescoby-Chambers N, Forster P, Bates G (2001): "Foetal valproate syndrome and autism: additional evidence of an association." Dev Med Child Neurol 43:847.
87. Lee LC, Newschaffer CJ, Lessler JT, Lee BK, Shah R, Zimmerman AW (2008): Variation in season of birth in singleton and multiple births concordant for autism spectrum disorders. Paediatr Perinat Epidemiol 22:172–179.
88. Williams K, Helmer M, Duncan GW, Peat JK, Mellis CM (2008): Perinatal and maternal risk factors for autism spectrum disorders in New South Wales, Australia. Child Care Health Dev 34:249–256.
89. Gardener H, Speigelman D, Burka S (2009): Perinatal risk factors for autism: Comprehensive meta-analysis. Br J Psychiatry 195:7–14.
90. Gardener H, Spiegelman D, Buka SL (2011): Perinatal and neonatal risk factors for autism: A comprehensive meta-analysis. Pediatrics 128:344–355.
91. Froehlich-Santino W, Tobon AL, Cleveland S, Torres A, Phillips J, Cohen B, *et al*. (2014): Prenatal and perinatal risk factors in a twin study of autism spectrum disorders. J Psychiatr Res 54: 100–108.
92. Zerbo O, Iosif AM, Walker C, Ozonoff S, Hansen RL, Hertz-Picciotto I (2013): 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 43:25–33.
93. Atladottir HO, Henriksen TB, Schendel DE, Parner ET (2012): Autism after infection, febrile episodes, and antibiotic use during pregnancy: An exploratory study. Pediatrics 130:e1447–e1454.
94. Atladóttir HO, Parner ET, Schendel D, Dalsgaard S, Thomsen PH, Thorsen P (2007): Time trends in reported diagnoses of childhood neuropsychiatric disorders: A Danish cohort study. Arch Pediatr Adolesc Med 161:193–198.
95. Patterson PH (2012): Maternal infection and autism. Brain Behav Immun 26:393.
96. Croen LA, Braunschweig D, Haapanen L, Yoshida CK, Fireman B, Grether JK, *et al*. (2008): Maternal mid-pregnancy autoantibodies to fetal brain protein: The early markers for autism study. Biol Psychiatry 64:583–588.
97. Singer HS, Morris CM, Gause CD, Gillin PK, Crawford S, Zimmerman AW (2008): Antibodies against fetal brain in sera of mothers with autistic children. J Neuroimmunol 194:165–172.
98. Enstrom AM, Van de Water JA, Ashwood P (2009): Autoimmunity in autism. Curr Opin Investig Drugs 10:463–473.
99. Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, *et al*. (2003): Maternal neuronal antibodies associated with autism and a language disorder. Ann Neurol 53:533–537.
100. Singer HS, Morris C, Gause C, Pollard M, Zimmerman AW, Pletnikov M (2009): Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: A pregnant dam mouse model. J Neuroimmunol 211:39–48.
101. Martin LA, Ashwood P, Braunschweig D, Cabanlit M, Van de Water J, Amaral DG (2008): Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism. Brain Behav Immun 22:806–816.
102. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, *et al*. (2011): Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474:380–384.
103. Schmidt R, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, *et al*. (2012): Maternal periconceptional folic acid intake and risk of autism specturm disorders and developmental delay in the CHARGE (CHilhood Autism Risk from Genetics and Environment) case-control study. Am J Clin Nutr 96:80–89.
104. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, *et al*. (2013): Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA 309: 570–577.
105. Sandin S, Hultman CM, Kolevzon A, Gross R, MacCabe JH, Reichenberg A (2012): Advancing maternal age is associated with increasing risk for autism: A review and meta-analysis. J Am Acad Child Adolesc Psychiatry 51:477–486. e471.
106. Idring S, Magnusson C, Lundberg M, Ek M, Rai D, Svensson AC, *et al*. (2014): Parental age and the risk of autism spectrum disorders: Findings from a Swedish population-based cohort. Int J Epidemiol 43:107–115.
107. D'Onofrio BM, Rickert ME, Frans E, Kuja-Halkola R, Almqvist C, Sjolander A, *et al*. (2014): Paternal age at childbearing and offspring psychiatric and academic morbidity. JAMA Psychiatry 71:432–438.
108. Lyall K, Schmidt RJ, Hertz-Picciotto I (2014): Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol 43:443–464.
109. Tsuang MT, Bar JL, Stone WS, Faraone SV (2004): Gene-environment interactions in mental disorders. World Psychiatry 3: 73–83.
110. Yang Q, Khoury MJ (1997): Evolving methods in genetic epidemiology. III. Gene-environment interaction in epidemiologic research. Epidemiol Rev 19:33–43.
111. Kendler KS, Gardner CO (2010): Interpretation of interactions: Guide for the perplexed. Br J Psychiatry 197:170–171.
112. Ahlbom A, Alfredsson L (2005): Interaction: A word with two meanings creates confusion. Eur J Epidemiol 20:563–564.
113. van der Mei IA, Otahal P, Simpson S Jr, Taylor B, Winzenberg T (2014): Meta-analyses to investigate gene-environment interactions in neuroepidemiology. Neuroepidemiology 42:39–49.
114. Rothman KJ, Greenland S (2008): Modern Epidemiology, 3rd ed. Philadelphia: Lippincott Williams & Wilkins.
115. Garcia-Closas M, Rothman N, Figueroa JD, Prokunina-Olsson L, Han SS, Baris D, *et al*. (2013): Common genetic polymorphisms

modify the effect of smoking on absolute risk of bladder cancer. Cancer Res 73:2211–2220.
116. Kendler KS, Eaves LJ (1986): Models for the joint effect of genotype and environment on liability to psychiatric illness. Am J Psychiatry 143:279–289.
117. Jaffee SR, Price TS (2007): Gene-environment correlations: A review of the evidence and implications for prevention of mental illness. Mol Psychiatry 12:432–442.
118. Fairchild G, van Goozen SH, Calder AJ, Goodyer IM (2013): Research review: Evaluating and reformulating the developmental taxonomic theory of antisocial behaviour. J Child Psychol Psychiatry 54:924–940.
119. Hunter DJ (2005): Gene-environment interactions in human diseases. Nat Rev Genet 6:287–298.
120. Merikangas K, Karayiorgou M (2014): Genetics of psychiatric disorders: Advances in genetic epidemiology and molecular genetics. In: Tasman A, Kay J, Lieberman A, First M, Riba R, editors. Psychiatry, 4th ed. New York: John Wiley & Sons.
121. Gatto NM, Campbell UB, Rundle AG, Ahsan H (2004): Further development of the case-only design for assessing gene-environment interaction: Evaluation of and adjustment for bias. Int J Epidemiol 33:1014–1024.
122. Albert PS, Ratnasinghe D, Tangrea J, Wacholder S (2001): Limitations of the case-only design for identifying gene-environment interactions. Am J Epidemiol 154:687–693.
123. Glasson EJ, Bower C, Petterson B, de Klerk N, Chaney G, Hallmayer JF (2004): Perinatal factors and the development of autism: A population study. Arch Gen Psychiatry 61:618–627.
124. Lavezzi AM, Ottaviani G, Matturri L (2005): Adverse effects of prenatal tobacco smoke exposure on biological parameters of the developing brainstem. Neurobiol Dis 20:601–607.
125. Xu Z, Seidler FJ, Ali SF, Slikker W Jr, Slotkin TA (2001): Fetal and adolescent nicotine administration: Effects on CNS serotonergic systems. Brain Res 914:166–178.
126. Slotkin TA, Tate CA, Seidler FJ (2006): Prenatal nicotine exposure alters the responses to subsequent nicotine administration and withdrawal in adolescence: Serotonin receptors and cell signaling. Neuropsychopharmacology 31:2462–2475.
127. Pletnikov MV, Rubin SA, Vogel MW, Moran TH, Carbone KM (2002): Effects of genetic background on neonatal Borna disease virus infection-induced neurodevelopmental damage. II. Neurochemical alterations and responses to pharmacological treatments. Brain Res 944:108–123.
128. Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, *et al*. (2011): Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology 22:476–485.
129. Volk H, Kerin T, Lurmann F, Hertz-Picciotto I, McConnell R, Campbell D (2014): Autism spectrum disorder interaction of air pollution with the MET receptor tyrosine kinase gene. Epidemiology 25:44–47.
130. Duncan LE, Keller MC (2011): A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. Am J Psychiatry 168:1041–1049.
131. Boffetta P, Winn D, Ioannidis JP, Thomas D, Little J, Smith G, *et al*. (2012): Recommendations and proposed guidelines for assessing the cumulative evidence on joint effects of genes and environments on cancer occurrence in humans. Int J Epidemiol 41:686–704.
132. Gauderman WJ, Morrison JM (2006): QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies.; Available at: http://biostats.usc.edu/software. Accessed June 12, 2014.
133. Stoltenberg C, Schjoberg S, Bresnahan M, Hornig M, Hirtz D, Dahl C, *et al*. (2010): The autism birth cohort: A paradigm for gene-environment-timing research. Mol Psychiatry 15:676–680.
134. Madsen KM, Hviid A, Vestergaard M, Schendel D, Wohlfahrt J, Thorsen P, *et al*. (2002): MMR vaccination and autism—a population-based follow-up study [in Danish]. Ugeskr Laeger 164: 5741–5744.
135. Tran PL, Lehti V, Lampi KM, Helenius H, Suominen A, Gissler M, *et al*. (2013): Smoking during pregnancy and risk of autism spectrum disorder in a Finnish National Birth Cohort. Paediatr Perinat Epidemiol 27:266–274.
136. Power RA, Kyaga S, Uher R, MacCabe J, Langstrom N, Landen M, *et al*. (2013): Fecundity of patients with schizophrenia, autism, bipolar disorder, depression, anorexia nervosa or substance abuse vs their unaffected siblings. JAMA Psychiatry 70:22–30.
137. Newschaffer CJ, Croen LA, Fallin MD, Hertz-Picciotto I, Nguyen D, Lee NL, *et al*. (2012): Infant siblings and the investigation of autism risk factors. J Neurodev Disord 4:1–16.
138. Committee on Human and Environmental Exposure Science in the 21st Century, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council (2012): Exposure Science in the 21st Century: A Vision and a Strategy. Washington, DC: The National Academies Press.
139. White E, Hunt JR, Casso D (1998): Exposure measurement in cohort studies: The challenges of prospective data collection. Epidemiol Rev 20:43–56.
140. Di Sclafani V, Finn P, Fein G (2008): Treatment-naive active alcoholics have greater psychiatric comorbidity than normal controls but less than treated abstinent alcoholics. Drug Alcohol Depend 98:115–122.
141. Berkson J (1946): Limitations of the application of the fourfold table analysis to hospital data. Biometrics 2:47–53.
142. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC) (2014): Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 63:1–21.
143. Abrahams BS, Geschwind DH (2008): Advances in autism genetics: On the threshold of a new neurobiology. Nat Rev Genet 9:341–355.
144. Constantino JN, Gruber CP (2005): Social Responsiveness Scale (SRS). Los Angeles: WPS.
145. Stamou M, Streifel KM, Goines PE, Lein PJ (2013): Neuronal connectivity as a convergent target of gene x environmental interactions that confer risk for Autsim Specturm Disorders. Neurotoxicol Teratol 36:3–16.
146. Balshaw DM, Kwok RK (2012): Innovative methods for improving measures of the personal environment. Am J Prev Med 42:558–559.
147. Kato N (2013): Insights into the genetic basis of type 2 diabetes. J Diabetes Investig 4:233–244.
148. Patel CJ, Chen R, Kodama K, Ioannidis JP, Butte AJ (2013): Systematic identification of interaction effects between genome- and environment-wide associations in type 2 diabetes mellitus. Hum Genet 132:495–508.
149. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, *et al*. (2014): Most genetic risk for autism resides with common variation. Nat Genet 46:881–885.
150. Vinkhuyzen AA, Wray NR, Yang J, Goddard ME, Visscher PM (2013): Estimation and partition of heritability in human populations using whole-genome analysis methods. Annu Rev Genet 47: 75–95.