



Gene-environment interaction and autism spectrum disorder

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Abstract

Introduction

In the following review, we will summarize the existing literature of population-based gene-environment interaction studies in ASD. In addition, we will suggest strategies and data sets that may be appropriate moving forward to perform these methodologically challenging studies.

Conclusion

Based on initial findings from only a handful of studies, combined with the strong biologic likelihood, researchers should increase their focus on gene-environment interaction.

Introduction

Gene-environment interaction has long been considered a likely genetic mechanism contributing to the risk for autism spectrum disorder (ASD)¹.

While ASD is one of the most heritable psychiatric disorders, demonstrated by concordance rates among monozygotic (MZ) twins ranging from 60-90%² and dizygotic (DZ) twins from 4.5%²-36%³, the lack of complete concordance among MZ twins precludes a simple genetic model. In the last few years evidence for a non-genetic contribution has been growing. In 2011, a study of 192 twin pairs estimated that genes account for 38% of ASD risk while environmental factors explain the remaining 62%⁴. It has been postulated that the range of concordances estimated for MZ twins, as well as the clinical heterogeneity of MZ twins may be explained by the difference in the uterine environment⁵. However, given the

high prevalence of many associated environmental factors, such as air pollution^{6,7}, gestational diabetes⁸, and maternal infections⁹ or fever¹⁰ in pregnancy, genetics and the environment clearly play a dual role. The exact nature of that role is an active area of research. A lack of replicable results from candidate gene studies and 'missing heritability'¹¹ has prompted hypotheses regarding potential genetic mechanisms that may better describe ASD risk, including gene-gene interaction, epigenetics and gene-environment interaction¹².

While few published studies have explicitly searched for and identified gene-environment interaction related to ASD risk, evidence thus far supports the involvement of gene-environment interaction. The joint gene-environment contribution that will be described in this review is the circumstance where a genetic effect exists in a subgroup (for example, smokers), but not or to a different degree in the other subgroup (non-smokers). Another mechanism by which the environment can modify a genetic effect is through epigenetics. Though the present review would be remiss to not discuss epigenetics, it has been recently reviewed in relation to ASD research elsewhere^{13,14}, and therefore will be covered only briefly.

Epigenetics includes heritable changes in gene expression that do not involve mutations in the DNA sequence, including DNA methylation and histone modification. While epigenetic changes can arise by stochastic processes, they are also influenced by the environment¹⁵ and do describe an additional mechanism of gene-environment interaction.

In the following review, we will summarize the existing literature of population-based gene-environment

interaction studies in ASD. In addition, we will suggest strategies and data sets that may be appropriate moving forward to perform these methodologically challenging studies.

Discussion

Studies of gene-environment interaction

Epidemiologic studies

An early study exploring gene-environment interaction in relation to ASD identified only indirect evidence of interactions¹⁶ (Table 1).

Serum paraoxonase, in combination with cytochrome P450, plays a major role in the detoxification of organophosphates¹⁷. The gene PON1 encoding the protein paraoxonase has known functional polymorphisms that affect the speed of organophosphate metabolism. Organophosphates are compounds that are widely used as pesticides in agriculture in both the United States and Italy.

They are also used as household insecticides, however their use for this purpose is far less in Italy compared with the United States. This led D'Amelio et al. to hypothesize that polymorphisms of PON1 would be associated with ASD in the United States, but not in Italy¹⁶. The authors performed both case-control association analyses of 177 Italian and 135 Caucasian-American individuals with ASD compared with 180 Italian and 376 Caucasian ethnically matched controls as well as family based linkage and association studies (177 Italian and 107 Caucasian-American families). As hypothesized, Caucasian-American, but not Italian patients, differed from controls comparing the Q192R SNP of PON1 (odds ratio (OR)=1.73, 95% confidence interval (CI) 1.12, 2.69).

Results were confirmed in transmission disequilibrium testing (TDT) analyses of complete trios identifying

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preferential transmission of the 192R alleles from Caucasian, but not Italian parents ($p < 0.025$). Family-based association tests (FBAT) analyses confirmed the association for Q192R and also identified a significant association with L55M ($p = 0.01$).

While these results do not provide direct evidence for gene-environment interaction, given the known environmental differences between the two populations combined with the genetic findings suggest that a combination of genetic variation and the environmental (organophosphates) may increase the risk for ASD.

Studies that followed provided the first direct evidence of gene-environment interaction in relation to ASD risk. In 2007, Cheslak-Postava et al. evaluated the association between SNPs in the beta-adrenergic receptor gene (ADRB2) and ASD in 331 case-parent trios from the autism genetic resource exchange (AGRE)¹⁸.

The ADRB2 encodes a catecholamine receptor which, once bound, stimulates adenylyl cyclase to convert ATP to cyclic AMP, a molecule important for cell replication, differentiation and survival¹⁸.

This study was motivated by a prior twin study which suggested prenatal overstimulation of the β 2-adrenergic receptor is a risk factor for ASD based on greater ASD concordance among DZ twins exposed to terbutaline as well as an association between two ADRB2 SNPs, selected based on in vivo evidence of their effect on responsiveness, desensitization and down regulation. The SNPs included a G for A substitution in codon 16 (rs1042713) and a G for C substitution at codon 27 (rs1042714).

Due to small numbers, the authors compared the frequency of the polymorphisms to reported population frequencies¹⁹. The authors of the motivating twin study suggested the polymorphisms may create a susceptibility to ASD that

is further influenced by terbutaline, however due to small number the authors were unable to evaluate interacting effects¹⁹. Therefore, Cheslak-Postava et al. tested the association between these two SNPs with ASD and additionally evaluated the combined effects of genetic variation and pregnancy-related stress. Marginal genetic effects were tested among 331 ASD case-parent trios, while gene-environment interaction was evaluated among a subset ($n = 144$) who had complete clinical data¹⁸.

Pregnancy-related stress served as a proxy for endogenous catecholamine exposure and was defined by the presence of one or more of the following: severe infection or fever, vaginal bleeding, generalized oedema, hypertension, albuminuria, gestational diabetes, eclampsia/pre-eclampsia, multiple pregnancies or an abnormal foetal screen.

While no association was observed between ASD and rs1042713, significant gene-environment interaction was observed between pregnancy related stress and rs1042714, such that the homozygotes glu27/glu27 (glutamic acid rather than glutamine) was associated with an OR=3.03 (95% CI: 1.15, 7.98) in the presence of maternal stress and OR=1.07 (95% CI: 0.35, 3.28) if no maternal stress was present¹⁸.

More recently two additional gene-environment interactions were identified within the case-control CHARGE (Childhood Autism Risks from Genetics and Environment) Study^{20,21}. In addition to a main effect association between maternal reported prenatal vitamins intake in the three months prior to pregnancy or the first month of pregnancy and ASD, Schmidt et al. identified a significant interaction between periconceptual prenatal vitamins and SNPs in genes involved in one-carbon metabolism. The genes that interacted significantly included 5,10-methylenetetrahydrofolate reductase (MTHFR) and catechol-O-

methyltransferase (COMT), each which had previously been associated with ASD and cystathionine-beta-synthase (CBS). The MTHFR gene has multiple roles in regulating folate availability and the 677 TT genotype is associated with 60% decreased enzyme activity. Among mothers who did take prenatal vitamins the OR was not elevated comparing the MTHFR TT to CC or CT genotype OR= 0.74 (95% CI: 0.36, 1.5). Similarly, comparing women who did not take prenatal vitamins to those who did take prenatal vitamins (all who were either CT or CC) the odds of having a child with ASD were not elevated OR=1.2 (95% CI: 0.77, 2.0). However, the combined effect of not taking prenatal vitamins and the TT compared with CT/CC genotypes did increase the odds (OR=4.5 (95% CI: 1.5,4, 14.6)²⁰.

The second maternal gene variation that interacted significantly was CBS. Though the effect of the GT/TT was null if mothers did take periconceptual prenatal vitamins, if they did not take vitamins the OR=2.6 (95% CI: 1.2, 5.4). While the prior to findings described variation in maternal genes (MTHFR and CBS), an association was also observed with a COMT variant in the child genome. With maternal vitamin intake and the AA genotype, (compared with GG or AG) there was an increased odds for ASD, though it did not reach statistical significance OR=1.8 (0.99, 3.5); however if the mother did not take vitamins there OR was appreciably higher and statistically significant association OR=7.2 (95% CI: 2.3, 22.4)²⁰. The final example of gene-environment interaction was observed between traffic-related air pollution exposure during pregnancy and the met proto-oncogene (MET).

The MET gene encodes a receptor tyrosine kinase that mediates hepatocyte growth factors signalling and is involved in formation of brain circuits, synapse formation, immune function and gastrointestinal repair²². The MET gene is highly correlated with protein levels and has been associated with ASD in several independent cohorts²³. In addition, cases with ASD

Table 1: Summary of studies evaluating the gene-environment interaction and ASD.

Author, year	Population (n, country)	Environmental factor	Gene and variation	Chromosome location*	Results
D'Amelio ¹⁶	177 Italian families (180 unaffected Italian controls); 108 Caucasian-American families. [additional 252 Canadian individuals GxG interaction]	Organophosphates	PON1 -C-108T -L55M -Q192R (variants associated with reduced serum paraoxonase or decreased affinity for OPs)	7q21.3-22.1	No SNPs significant in Italian samples, but PON1 L55/R192 (compared with M55/Q192) significant in American families
Cheslak-Postava ¹⁸	n=331 ASD case-parent trios (AGRE), USA Subset with clinical data to define environmental component: n=144	Pregnancy-related stress as proxy for endogenous catecholamine exposure (severe infection or fever, vaginal bleeding, generalize edema, hypertension, albuminuria, gestational diabetes, eclampsia/pre-eclampsia, multiple pregnancy or abnormal fetal screen.	ADRB2 -rs1042713 (codon 16) -rs1042714 (codon 27)	-5q31-32	-No interaction -Glu27/Glu27 homozygous OR 3.03 (1.15, 7.98) in presence of maternal pregnancy stress; OR=1.07 (0.35, 3.28)
Schmidt ²⁰	n=232 typically developing families n=238 ASD families (included Hispanic and non-Hispanic Black, Asian and mixed race) California	Periconceptional maternal prenatal vitamin intake	-MTHFR 677T (maternal) -CBS rs234715 (maternal) -COMT472AA	-1p36.3 -21q22.3 -22q11.21	-OR=4.5 (1.4, 14.6) -OR=2.6 (1.2, 5.4) -OR=7.2 (2.3, 22.4) Comparable results with sensitivity analyses restricted to non-Hispanic white.
Volk ²¹	Total n=408, of those n=252 ASD (age 2-5) *same case-control study as Schmidt et al.	Traffic-related air pollution and particulate matter (PM) (<2.5 and <10 microns), nitrogen dioxide, and ozone	MET -rs1858830	7q31	Higher risk for children with MET variant (CC) and high air pollution exposure compared to those without variant MET (CG/GG) and low pollution exposure -traffic related (OR=2.9 (1.0, 10.6) -PM ₁₀ (OR=3.2, 1.3, 9.1) -Nitrogen dioxide (OR=3.6 (1.3, 12.7)

*Chromosomal regions not provided in the paper were identified through NCBI Gene search

demonstrate reduced MET protein expression compared with age- and sex-matched controls²².

Epidemiologic studies identified an association between ASD and air pollution exposure during pregnancy or early life^{6,7}. Initial evidence of

interaction between air pollution and MET arose from a mouse study evaluating the interaction between in utero benzo(a)pyrene (BAP), a polycyclic aromatic hydrocarbon (PAH) that is a component of both traffic pollution and particulate matter, and the MET gene²⁴. Pregnant

mice were exposed BAP based on a susceptibility-exposures paradigm to replicate exposure during key periods of brain development. This exposure lead to a decrease in MET protein expression as well as altered behavior in the offspring. Building on this prior work, Volk et al. compared 252 cases

Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.



with ASD were to 156 typically developing controls from the CHARGE study²¹. The authors observed that variation in SNP rs1858830 of the child's MET gene (comparing MET CC genotype with the CG/GG) was not associated with ASD among children with low air pollution exposure.

However, there was a 3-fold increased odds for ASD for children carrying the high risk gene variant (CC) who also had high prenatal exposure to local traffic-related air pollution (aOR=2.9, 95% CI: 1.01, 10.6), regional particulate matter (PM10) (aOR=3.2, 95% CI: 1.3, 9.1) and nitrogen dioxide exposure (aOR=3.6, 95% CI: 1.3, 12.7) compared with those who had low exposure and did not carry the high risk genotype²⁵.

Future research

Each of the studies reviewed reported evidence of gene-environment interaction, suggesting this is a promising area for future research in ASD. There are some caveats however; for example, it is difficult to know whether the results represent a publication bias resulting from a failure to publish null results. In fact, a critical review of the literature describing candidate gene and environment interaction in psychiatry did find evidence of publication bias as most of the initial publications were significant, while only 27% of the replication studies identified significant interaction²⁶. Despite the possible existence of publication bias and given the high biologic plausibility, the environmental context should be considered in genetic studies moving forward.

Two ongoing studies may provide excellent resources for exploring gene-environment interaction include The National Children's Study²⁷ and the Early Autism Risk Longitudinal Investigation (EARLI)²⁸. The National Children's Study aims to enrol 100,000 children in a nationally representative, longitudinal study that follows children from birth to conception. Specific strengths for gene-environment inquiry include the

large sample size and array of non-genetic exposure measures including chemical, physical biological and psychosocial).

EARLI is a large prospective birth cohort that includes families with a child ASD and who are therefore at risk for having another child with ASD. The EARLI study was specifically designed to identify pre-, peri- and neonatal risk factors that may interacted with a genetic predisposition.

The EARLI study has several strengths enabling the detection of interaction including, the prospective nature of the environmental exposure measurements (increasing temporal sequencing), the enriched-risk pregnancy cohort study to increase power based on a higher number of outcomes than expected in the general population, and comprehensive biomarker collections for genetic analyses. Each of these studies will provide insight into the joint gene-environment contribution.

A major obstacle for studies exploring any type of interaction (gene-gene or gene-environment) is power. While the scale of the National Children's Study may provide a large sample for interaction studies, additional studies of this size, especially for an independent researcher, are not feasible. In general, prospective cohort studies are not an efficient study design for rare disorders. In addition, there are large costs to genotyping and collecting environmental data on so many people. New and innovative methods will be necessary to leverage existing genetic and environmental resources.

For example, the electronic Medical Records and Genomics (eMERGE) Network, is consortium of institutions that have combined DNA repositories with electronic medical records that provide a litany of clinical and demographic data²⁹. In addition, genetic resource, such as eMERGE, can be linked to environmental resources, such as state maintained vital records and environmental toxicant

monitoring (or air pollution or drinking water). When personal identifiers are available similar methods could be used for large genetic consortiums, such as the Autism Genome Project (AGP).

Conclusion

Based on initial findings from only a handful of studies, combined with the strong biologic likelihood, researchers should increase their focus on gene-environment interaction. While the oversimplification of the nurture versus nature debate was acknowledged years ago, genes and environment are rarely considered together.

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Competing interests: None declared. Conflict of interests: None declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
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Competing interests: None declared. Conflict of interests: None declared.
 All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
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