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Autism and Environmental Genomics: Synergistic Systems Approaches to Autism Complexity

Points of Interest

- Genomic and environmental factors work together in determining risk and severity of ASDs. Four mechanisms are highlighted:
  - Gene–environment interactions (GxE) (genetic susceptibility to environmental exposures).
  - Environmental factors causing genetic damage in germ cells (including point mutations or structural changes).
  - Environmental factors acting via heritable epigenetic modifications.
  - Genetic traits influencing environmental exposure via behavior (sometimes called *gene–environment correlation*).
- The apparently high heritability of ASDs is often misinterpreted as ruling out a large role for environmental risk factors. In fact, common, preventable environmental factors might be just as necessary as genetic factors for the occurrence of ASDs, despite high heritability, because of gene–environment interplay:
  - Heritability estimates mistakenly count gene–environment interaction as purely genetic for environmental exposures shared by twins.
  - Many genetic contributors may actually depend upon environmental exposures to have an impact, making the exposures the root cause.
  - Heritable epigenetic causes also may result from environmental exposures as the root cause.
  - Shared placentas are an environmental factor that may boost monozygotic (MZ) twin concordance and apparent heritability (through a different sort of gene–environment correlation). Genetic effects on a child's individual (nonshared) social environment (traditional gene–environment correlations) also inflate apparent heritability.
- Genetic findings in ASDs should inform research on environmental pollutants (or other risk factors) and vice versa, since genes and pollutants may have common targets. Greater collaboration, synthesis, and prioritization of risk factors based on toxicology and population attributable fraction (PAF) would be valuable. A systems and pathway-based approach, using bioinformatics and toxicogenomics tools would also benefit ASD research.

Introduction

What is "environmental genomics" and why is it important in research on autism spectrum disorders? The title of this chapter is meant to suggest that both environment and genetics are important in ASDs, and that studying the genome and environment together, in an integrated, systems approach, can drive a fruitful research and intervention agenda.

Genetics is obviously important in ASDs. But the investigation of genetic influences has run into frustrating limitations, including a realization that genome-wide association studies suffer from a high rate of false positive findings (Ioannidis, 2005; Moonesinghe et al., 2007), as well as generally modest-to-small odds ratios (typically less than 1.5) (Wellcome Trust Case Control Consortium, 2007; Allen et al., 2008; Harrison & Weinberger, 2005). In cases where larger odds ratios are suggested in autism gene candidates, the risk-associated variants are rare, so the PAF is still small, meaning that no single genetic factor has been able to account for even 50% of all cases of ASDs. Even all de novo copy number variants (CNVs) combined may be a critical factor in roughly 6% (Sebat et al., 2007) or speculatively perhaps up to 30% or more of ASDs (Zhao et al., 2007; Guilmatre et al., 2009). Genetics research is in need of new approaches that can explain and ultimately treat or prevent a larger share of all cases of ASD.

A simple focus on environmental factors alone is likewise unable to provide an adequate explanation for autism. Although various lines of evidence implicate environmental risk factors in ASDs (Institute of Medicine, 2008; Newschaffer et al., 2007; Lathe, 2006; Pessah & Lein, 2008) no specific environmental factor as yet has been verified with a large PAF. This may be because each important environmental factor mainly affects some genetic subgroup, or because many environmental factors converge upon common biological targets; either would make detection of specific culprits unlikely, because (partly resulting from sample size requirements) almost no studies of environmental factors in autism have examined risk stratified by genetic subgroup or biological mechanism of impact.

Although many research groups are still focused on either genes or environmental risk factors alone, striking progress has been made by those who study how genome and environment work together to cause disease. As explained in this chapter, they work together in several important ways, and four mechanisms are highlighted here:

- Environmental factors causing genetic damage in germ cells (including point mutations or structural changes).
- Environmental factors acting through epigenetic modifications.
- Genetic traits influencing environmental exposure via behavior (sometimes called *gene–environment correlation*).
- Gene–environment interactions (GxE).

These four general mechanisms cover a broad range of potential routes by which environmental and genetic or epigenetic factors may work together to either cause or affect the severity and phenotypic diversity of complex conditions such as ASDs. These four types of causal routes may provide a useful overarching framework for considering the many specific mechanisms that may contribute to the risk and severity of various aspects of ASDs. The advantage of this simple framework is that it calls attention to a very broad range of ways in which environmental and inherited risk factors may jointly contribute to ASDs. In particular, this perspective extends beyond narrowly defined gene–environment interaction (as discussed below), to highlight the potential importance of environment in causing de novo genetic and also epigenetic alterations, both of which have received little attention in ASD research until very recently.

There has been substantial confusion in the general public about the relative importance of environment and genetics in autism. A critical factor underlying the debate has been the widespread citation of extremely high heritability estimates. In the face of these estimates many have considered the residual role for environmental factors to be so small as not to merit serious attention. However, upon examination it becomes apparent that these heritability estimates, as well as related estimates of PAF, can be misleading and lead to erroneous conclusions about the contributions of genetics and environment (Visscher et al., 2008). In general, there is a long history of misinterpretation of estimates of the share of cases attributable to environmental (or other) causes, as emphasized at the start of the authoritative textbook, *Modern Epidemiology*, by Rothman and Greenland (1998). As these authors point out,

> "There is a tendency to think that the sum of the fractions of disease attributable to each of the causes of the disease should be 100%… it is clear on a priori grounds that 100% of any disease is environmentally caused…. Similarly, one can show that 100% of any disease is inherited…. Many researchers have spent considerable effort in developing heritability indices that are supposed to measure the fraction of disease that is inherited. Unfortunately, these indices only assess the relative roles of environmental and genetic causes of disease in a particular setting."

The significance of this point is further developed later in this chapter, but the essential point is that a disease can be simultaneously 100% genetic and 100% environmental. Another way of putting it is that genetics may be essential, but not sufficient, to cause autism. An implication for public health is that if environmental factors are also essential, then environmental changes may be able to prevent disease or reduce its severity.

The mechanisms by which genome and environment work together in ASDs are still unclear for the most part. What is clear, however, is that the simplistic dichotomy of nature versus nurture is largely obsolete, and that both are essential.

Just as viewing genome and environment separately limits progress, a piecemeal approach to risk factors and biological avenues of inquiry may be insufficient. Given the limited findings from attempts to find single strong causes, shifting to a systems biology perspective (Kitano, 2002) (i.e., genomics and epigenomics rather than genetics, and multiple environmental factors rather than one chemical at a time) may allow us most effectively to synthesize and reconcile findings related to multiple genes and multiple environmental risk factors that seem disparate and inconsistent when considered separately. It is important to note that this does not mean ASDs should be treated as a monolithic disorder whose many and varied features can be explained by a unified set of causes. On the contrary, it is essential to recognize the heterogeneity of ASDs' features and causes (Happé et al., 2006), and a key goal should be synthesis at the population level, where various features are influenced by various causes across various individuals, forming a complete picture that accounts for the wide range of conditions.

Finally, autism research also would benefit from greater integration of findings across biological levels and areas of expertise, linking new data from genetics, toxicology, physiology, epidemiology, and other fields. The next phase of progress will require greater application of high-throughput data along with tools from systems biology and bioinformatics, to study more comprehensive sets of molecules, genomic features,

microbiota, physiological functions, cell types, brain regions, systems, and symptoms.

Part One: Mechanisms by which Genome and Environment Work Together

To help transcend the historical polarization, it will be useful to consider four ways environment and genome/epigenome can work together. These four causal routes are sketched in Figure 49.1.

Genetic Damage (E→ G→ D)

Some recent evidence has suggested that a substantial percentage of cases of autism—perhaps on the order of 6%, though some have suggested the majority of cases—may be attributable to de novo genetic changes (Sebat et al., 2007; Zhao et al., 2007). To the extent this is true, it becomes critical to determine the cause of these genetic alterations. Many authors have referred to de novo genetic changes as "sporadic" or as resulting from "stochastic processes." This may imply they occur for no particular reason or with no identifiable cause. But in fact environmental factors can cause genetic damage, and susceptibility to such damage is also influenced by nutrition (Bagnyukova et al., 2008) and by genetic factors, including genes involved in xenobiotic metabolism (Dorne, 2007) and DNA repair (Spry et al., 2007). Particularly if a substantial share of autism is attributable to de novo changes, this has important implications for etiology and possibly prevention.

DNA damage can be passed on to the next generation if it occurs in germ cells (sperm or oocytes), rather than just in somatic cells (as in cancer). The effects of germ line damage are initially seen as de novo mutations; but in subsequent generations, assuming the carrier reproduces, they are inherited and seem to be simply genetic causes of disease even if they originally resulted from environmental exposures.

Recent work suggests oxidative damage is a major cause of increased recombination and mutation in germ cells (Ohno et al., 2006). This hypothesis has profound implications for the study of genetic and environmental causes of disease, and suggests the need for research focused on environmental agents causing oxidative stress and subsequent DNA damage.

Numerous environmental factors result in DNA damage, including environmental toxicants, infectious agents, radiation, and some medications (Elespuru & Sankaranarayana, 2007). Given reported associations of ASDs with urban birth (Lauritsen et al., 2005; Williams et al., 2006), the link between urban air pollution and DNA damage may be relevant. Although hundreds of genotoxic chemicals are found in urban air samples, the components most often studied and implicated as causing DNA damage are components of particulate matter (a complex mixture of substances), particularly polycyclic aromatic hydrocarbons (PAHs) (DeMarini & Claxton, 2006; Tovalin et al., 2006). The urban environment, however, contains thousands of chemicals in addition to air pollutants, and a review of these candidates is beyond the scope of this discussion. The urban environment also differs from rural settings in terms of exposures to infectious agents and allergens in

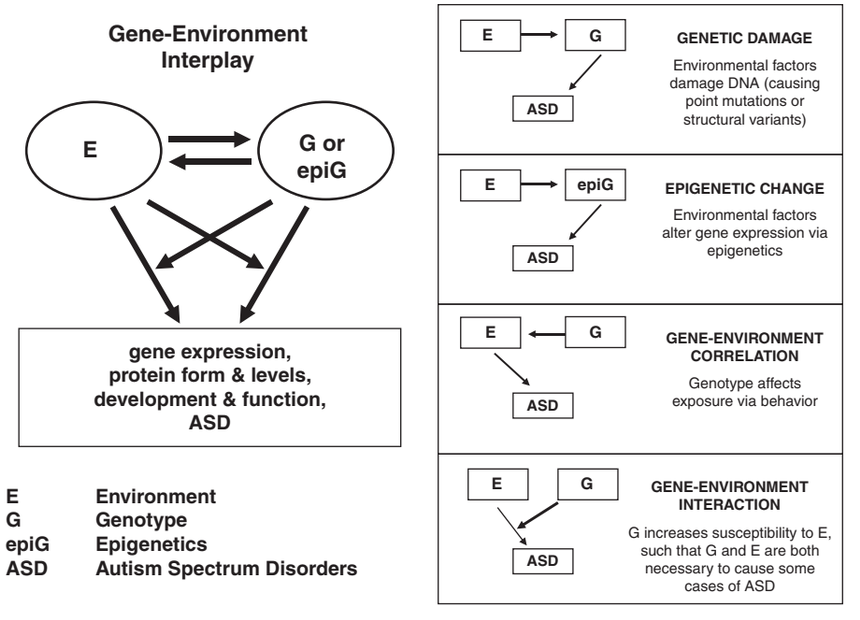


Figure 49–1. Potential Direct and Interactive Effects of Environment, Genes, and the Epigenome on Disease.

ways that increase risk, for example of allergies among certain genotypes (Becker, 2007; Martinez, 2007).

There are substantial gender differences in germ cell mutagenesis, in type and risk of mutation, which in cases of de novo mutations may provide clues about which parent was exposed and at what age (Crow, 2000; Eichenlaub-Ritter et al., 2007). Paternal age is a risk factor for ASDs (Croen et al., 2007; Kolevzon et al., 2007; Grether et al., 2009), and genetic damage in sperm cells does increase with the father's age (Wyrobek et al., 2006). Some recent research, though not specific to ASDs, suggests that >80% of de novo structural chromosomal abnormalities in live births are paternally derived, and most spontaneous point mutations also are of paternal origin, according to work cited by Eichenlaub-Ritter, Adler, Carere, and Pacchierotti (2007). Maternal age is a risk factor for aneuploidy (Hassold et al., 2007). Although full-blown aneuploidy does not appear to play a major role in ASDs, in one recent study 16% of 116 boys with idiopathic autism reportedly had greatly elevated rates of mosaic aneuploidy (mostly gains of the X chromosome) (Yurov et al., 2007).

Although dozens of germ line genotoxins have been identified in animal models, definitive identification of germ line mutagens in humans has been elusive for a variety of reasons (Wyrobek et al., 2007). The strongest evidence suggesting environmentally caused germ line genotoxicity in humans includes recent studies showing increased chromosomal aberrations and other forms of DNA damage in sperm following exposure to chemotherapeutic agents or radiation, and germ line mutations found in children born in heavily polluted areas following the Chernobyl accident (Wyrobek et al., 2007). Germ cell mutagens are defined and classified by the United Nations globally harmonized system (GHS) and other national approaches (Morita et al., 2006). There are well over 100 substances classified by the GHS as *Class 1B germ cell mutagens*, which are chemicals that "should be regarded as if they produce heritable mutations in the germ cells of humans." (United Nations, 2003)

A substantial body of evidence beginning in 1975 has shown that urban outdoor air pollution causes DNA damage, and more recent work has extended findings to heritable (germ line) mutations in male mice (Somers et al., 2004; Samet et al., 2004), with lower rates of DNA damage in rural areas or when urban air is first filtered to remove fine particulate matter, and these findings have been reviewed recently (Claxton & Woodall, 2007). Interestingly, DNA damage in human sperm now has been linked to elevated levels of air pollution, particularly in a high-risk GSTM1 genotype (Rubes et al., 2007), such that the preceding 90-day-average particulate matter concentration predicted elevated sperm DNA damage. Additional environmental factors including folate intake may affect susceptibility to germ line DNA damage (Boxmeer et al., 2008).

Environmental genotoxins can cause various types of point mutations, structural changes (deletions, insertions), or aneuploidy, through a variety of mechanisms (Salnikow & Zhitkovich, 2008; Schins & Knaapen, 2007; Husgafvel-Pursiainen, 2004). Different types of environmental mutagens cause differing types of DNA changes, and to some extent leave signatures. For example, as discussed in the very useful review by Claxton and Woodall (2007), G → T base substitutions are the main type of mutation produced by PAHs and nitroarenes. Research may be able to identify certain types of DNA alterations that occur more often in autism, and this might provide clues regarding the causes of those alterations. A recent study demonstrated the great potential of genome-wide analysis of various types of genetic damage and their connection with disease, in a comprehensive analysis of multiple tumors in human breast and colorectal cancer (Wood et al., 2007), where the two cancer types had different types of mutations, interpreted as suggesting exposure to different mutagens or differences in the DNA repair process.

Epigenetic Change/Damage (E→ epiG→ D)

Inherited epigenetic (epiG) alterations may be one explanation for why genetic factors identified to date have been unable to explain most cases of ASD, despite high heritability (although rare or multiple interacting factors are also possible explanations). In fact, it is very possible that the careful regulation of gene expression and more importantly protein levels, not the DNA sequence itself, is what really matters most often in ASDs. In any event, it is increasingly recognized that gene or protein dosage, not just function, can be critical in neurodevelopment.

Epigenetic change and damage in ASDs have thus far been little studied, so their potentially important roles have not yet been elucidated. A significant role for epigenetic factors in autism has been proposed in recent years (Jiang et al., 2004), and is supported by several findings related to MECP2 and MBD1 (Hogart, 2007; Cukier et al., 2008; Allan et al., 2008; Nagarajan et al., 2006), as well as evidence that methylation is impaired in some fraction of ASD cases (James et al., 2006; Deth et al., 2008) and possibly the fact that valproic acid (a risk factor for autism and used to create an animal model of autism) causes epigenetic changes, via its action as a histone deacetylase (HDAC) inhibitor (Schneider et al., 2008; Moore et al., 2000; Khan et al., 2008). Genetic disruptions of normal epigenetic mechanisms also appear to be important in causing mental retardation (Kramer, & van Bokhoven, 2008), and their importance in the nervous system has been reviewed (Colvis et al., 2005). Knowledge is rapidly evolving in this area, and it must be noted that the practical significance of these environmental influences on the epigenome in human health and disease overall remains to be established.

A number of environmental factors are known to alter DNA methylation patterns, including certain dietary factors, such as the intake of methyl donors (folates, choline, methionine), and DNA methyltransferase inhibitors (e.g., polyphenols and perhaps isothiocyanates from plants) (Johnson & Belshaw, 2008; Edwards & Myers, 2007). Furthermore, environmentally caused genetic damage can disrupt mechanisms of epigenetic control in some cases: oxidative damage to methyl-CpG sites

on DNA has been shown to impair the ability of MECP2 to bind to these target sites (Valinluck et al., 2004).

We also note that microRNAs and other forms of ribonucleic acid (RNA) are another aspect of the genome that is relatively understudied but may be important in mediating the effects of environment (Service, 2008; Riddihough et al., 2008; Bao et al., 2007) and in ASDs specifically (Abu-Elneel et al., 2008). For example, the 15q11 to 15q13 autism locus includes a locus coding for a small nucleolar RNA (snoRNA) that regulates serotonin 2c receptor splicing (Kishore & Stamm, 2006).

A group of major studies of cancer genomics has revealed that epigenetic changes, point mutations, and copy number variants all contribute to disruption of key pathways in various types of tumors (Jones et al., 2008; Parsons et al., 2008; Chan et al., 2008; The Cancer Genome Atlas Research Network et al., 2008; Wood et al., 2007). Of particular note, the mechanism of disruption varied across tumors and genes, sometimes involving point mutations, and in other cases CNVs or altered methylation. A pathway-based, comprehensive analysis including epigenetics was essential to illuminating all relevant genes and pathways.

Strikingly, some similar findings have been reported in ASD research. Different types of defects may alter MECP2 protein levels or function in different cases, including genetic (exon or promoter mutations) and epigenetic (promoter hypermethylation) (Nagarajan et al., 2006). One study of protein and mRNA expression in frontal cortex tissue from autistic and other patients revealed that multiple pathways, including apparently both transcriptional and posttranslational mechanisms, account for variation in expression of different MECP2 transcripts within different neuronal subsets (Samaco et al., 2004). Epigenetics rather than genetics explained the majority of cases of reduced MECP2 expression in autism cases in this study, although only 14 autism cases were analyzed. More recently, a similar pattern has been observed for the oxytocin receptor gene, where promoter methylation was increased in ASD cases relative to controls, in addition to some cases of deletions involving this gene (Gregory, 2009).

A further complexity is that some individuals or families are genetically predisposed to greater epigenetic stability versus lability over time in response to environment: the amount of change in global methylation over an 11- or 16-year interval clustered within families (Bjornsson et al., 2008). This might result in a gene–environment interaction in which certain gene variants make an individual's epigenome more susceptible to environmental exposures, resulting in substantially increased risk of disease when such gene variants and exposures co-occur.

Epigenetics is a relatively new field, and the mechanisms and implications are still being explored. Epigenetic changes can act like a strange hybrid of genetic and environmental risk factors, in that they can reflect the effects of the environment but also be inherited as relatively stable multigenerational factors. This complicates the way genes and environment work together to cause disease, and is not accounted for in most models of heritability or environmental risk. If epigenetic changes are actually a frequent cause of ASDs, this will have major implications for the types of genetic/epigenetic and environmental studies that should be undertaken. Resources are becoming available to investigate these issues more comprehensively, including the Human Epigenome Project, which has been attempting to catalog genome-wide DNA methylation patterns in all major tissues (Brena et al., 2006). Genome-wide epigenetic scans are becoming feasible (Berman et al., 2009), and it is clear they will add significantly to our understanding of the full range of causes of certain diseases.

Gene–Environment Correlation (G→ E→ D)

Gene–environment correlation is the situation where genotype affects one's social environment or one's exposure to environmental risk factors. To the extent that genes influence social environment or environmental exposures, it is possible that some genes increase the risk of autism by acting via environment. In other words, environment may be mediating some of the effects of genetics on autism risk. This is an underrecognized but important route of environmental influence, and one that needs to be understood in order to appropriately interpret heritability.

Genes can influence a child's exposure to environmental factors through various mechanisms, as discussed by Scarr and McCartney (1983) and Purcell (2002). There are at least three general routes by which genes could affect a child's social environment and exposure to physical/chemical environmental factors: *(1)* parental genes affecting parental behaviors that determine social and physical environmental exposure, *(2)* the child's genes affecting the child's behaviors, altering exposure to the social and physical environment, and *(3)* the child's genes affecting the child's behaviors, in turn affecting parental behavioral responses and the social environment.

Gene–environment correlation may be important to consider in autism research. Failure to take it into account can result in incorrect estimates of heritability and an incomplete picture of the prospects for intervention. If a genotype increases risk of autism, it is generally assumed that complete prevention is impossible, at least without gene therapy. However, if the genotype is actually causing autism partly by influencing the child's environment, interventions may be able to alter that environment and prevent some or all of the harm. For example, interventions focused on parenting practices (i.e., the social environment) can improve ADHD outcomes (Nigg, 2006), despite estimated heritability of 60% to 70% or even 90% (Nigg, 2006) and twin studies that identify almost no effects of shared environment. Gene–environment interactions and/or correlations could explain such seemingly conflicting findings.

Gene–Environment Interaction (GxE → D)

The term *GxE* is used here as shorthand for gene–environment interaction. The mechanisms underlying GxE, and even the

basic definition of what is meant by GxE, are still unclear in many cases. The term *gene–environment interaction* has been used in a variety of ways, sometimes referring to biological interactions and in other cases describing statistical interactions, for example (Khoury et al., 1993). As used here, GxE refers to genetic susceptibility, where the effects of environmental exposures differ depending on genotype. Of particular interest is the case where an added environmental exposure increases the risk of disease more in a high-susceptibility genotype subgroup than in the low-susceptibility genotype.

It has become very clear from research in a variety of fields that genetics and environment clearly can and do interact (Rieder et al., 2008; Kelada et al., 2003), including in some neurodevelopmental diseases (Genc & Schantz-Dunn, 2007; Caspi & Moffitt, 2006; Thapar et al., 2007; Tsuang et al., 2004).

The possibility of gene–environment interaction in ASDs has been raised (Lawler et al., 2004; Newschaffer et al., 2002). Hints of such interaction may be starting to emerge in autism research, such as in findings related to PON1 (Pasca et al., 2008; D'Amelio et al., 2005), glutathione-related factors (James et al., 2006), immune system alterations (Enstrom et al., 2008; Ashwood et al., 2008), and pathways involving the MET gene (Campbell et al., 2008).

GxE may be an important concept in ASD research for a variety of reasons:

- Studying GxE may help the field progress more rapidly in finding larger relative risk (RR) values and perhaps larger PAF values (Rutter, 2008).
- Studying GxE may identify important genes and medical intervention targets that would have been overlooked otherwise.
- Identifying environmental factors would be useful because some are avoidable or preventable in the near term, in contrast with genetic risk factors.
- Identifying genetic factors that create susceptibility to environment may enable targeted prevention efforts focused on a genetic subpopulation.

Table 49–1.
Nomenclature Used to Refer to Four Combinations of Genotype and Environmental Exposure

Relative Risk (vs. Baseline, Which is Low-Risk Genotype and no Exposure)	g: (Genotype A: Low Sensitivity to E)	G: (Genotype B: High Sensitivity to E)
E (exposed to environmental risk factor)	**gE (E has some direct effect, even in low-risk genotype)**	**GE (GxE means that RR is much larger among those with the high-risk combination of G and E)**
e: (not exposed)	**ge (baseline: no increased risk)**	**Ge (gene has some direct effect, even without E)**

Two simple scenarios may demonstrate how genes and environment can interact to increase risk of disease. Table 49.1 shows the nomenclature used to refer to four combinations of genotype and environmental exposure, and Figure 49.2 illustrates two simple scenarios. It is critical to note that if E is ubiquitous, both scenarios appear to be simple cases of genetic causation.

Scenario 1: Gene and environment are both necessary (Figure 49.2A).

The simplest type of GxE is the case where both G and E are necessary for any impact on risk (Scenario 1).

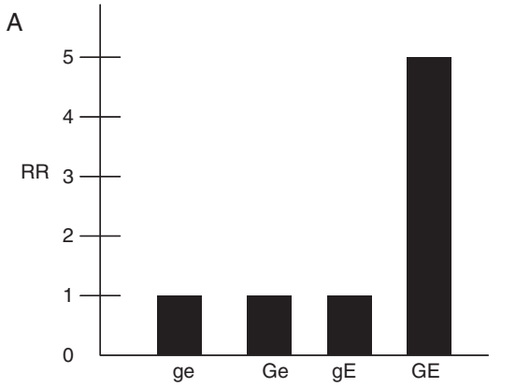
Scenario 2: Gene simply increases susceptibility to environment (Figure 49.2B).

In this case, the risk allele (G) alone (without E) has no effect, but E alone (in the low risk genotype, g) has some effect. The combination of G and E has a much larger effect on risk than E with the low-risk genotype (g). If, for example, G controls metabolism of a toxicant, in that capacity it has no effect on ASD risk on its own, and thus it only matters when E (the toxicant) is present. E has some modest impact on ASDs in the low-risk genotype (g), but much more effect in the high-risk genotype (G).

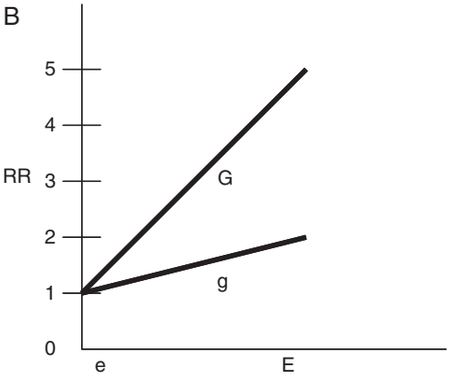
There are, in fact, several general types of GxE, with typologies proposed by Ottman (1990, 1996) and Khoury et al. (1988). An extensive discussion of gene–environment research, including study designs and other key issues, was provided in the report, *Genes, Behavior, and the Social Environment: Moving Beyond the Nature/Nurture Debate* (National Research Council, 2006). Standard nomenclature denoting various combinations of genotype and exposure has been applied in the context of risk analysis (Cullen et al., 2008). In some cases, G and E each have direct effects on risk, apart from their interaction; in other cases only G has direct effects. Impacts on risk might be nonlinear or even nonmonotonic. It is also important to distinguish between interaction on additive and multiplicative scales. The nature of the interaction in GxE also can be expressed in the "component causes" framework (Greenland & Robins, 1986; Rothman & Greenland, 2005). These complexities have been well-covered elsewhere, albeit not in the context of autism research (National Research Council, 2006).

Toxicokinetics and Toxicodynamics

A critical research goal is to elucidate the types of mechanisms underlying GxE. It may be useful to consider two broad areas where GxE can arise: toxicokinetics (TK) and toxicodynamics (TD). Toxicokinetic variability refers to differences between individuals in the extent to which an external dose (ambient environmental concentration outside the body) results in a biologically available dose at the target organ(s). Toxicokinetics involves absorption, distribution, metabolism, and elimination



RR	g	G
E	gE: RR = 1	GE: RR >>1
e	ge: RR = 1	Ge: RR = 1



RR	g	G
E	gE: RR = 2	GE: RR = 5
e	ge: RR = 1	Ge: RR = 1

Figure 49–2. Scenarios for the Interaction of Gene and Environment on Disease Risk. Explanation in Text.

(ADME) of toxicants and their metabolites. Toxicokinetics has been studied intensively in the field of toxicology, and numerous models have been developed to quantify interindividual TK variability, describing how some individuals receive a larger effective internal dose for the same external exposure. Toxicodynamics, in contrast, refers to the extent to which the available dose at the target organ(s) results in adverse effects on health.

The vast majority of genes studied in autism so far are not related to TK vulnerability, partly because candidate gene studies have prioritized "brain genes" rather than genes that might result in the brain being exposed to higher levels of environmental agents or vulnerable at lower levels of exposure (Herbert et al., 2006). Research and data in TK variability could inform the search for and analysis of potential environmental risk factors in ASDs. Research on autism's relationship with the PON1 gene that is important for organophosphate metabolism is one example (D'Amelio et al., 2005), and ASDs have been associated with two genes involved in metal metabolism (MTF1 and SLC11A3) (Serajee et al., 2004), but many other opportunities remain untapped. It is known that TK differences across the population can be caused by age, health status, infection and immune status, and genetic differences in a range of TK genes.

Genes that regulate metabolism of environmental chemicals

Numerous genes are thought to affect sensitivity to xenobiotics by altering metabolism of various compounds. These are reviewed relatively comprehensively and quantitatively by Dorne (2007).

Genetically sensitive individuals may have a given biological response or risk associated with a much lower ambient concentration of a chemical, compared with the average individual. Viewed in another way, genetically sensitive individuals may be at substantially higher risk than the average individual, when exposed to a given ambient concentration. TK variability in some major metabolic pathways may result in a twofold to even a tenfold or sometimes much greater increase in the effective dose experienced by individuals at the high end of the population distribution of TK variability (Dorne, 2007). TD factors would provide additional variability, resulting in even greater sensitivity for some individuals.

Genes relevant to infection: There are numerous genes known to control susceptibility to infection (Kaslow et al., 2008). If infection (or the maternal immune response to infection) is a risk factor for autism, then immune defense genes may be an important determinant of autism risk. This is an avenue of investigation that appears to be untapped in autism research.

Genes involved in DNA repair: Approximately 150 human genes involved in DNA repair have been identified, and mutations in many of these are known to increase the likelihood of DNA damage and cancer (Spry et al., 2007).

Genes upregulated or downregulated in response to environmental exposures: A recent review summarized gene expression changes seen in response to multiple air pollutants, including particulate matter, diesel exhaust, secondhand smoke from tobacco, and others. Key categories of genes upregulated by exposure to multiple air pollutant mixtures included genes related to xenobiotic metabolism (e.g., CYP1B1), oxidative stress response (e.g., glutathione-related genes, heme oxygenase 1, metallothioneins), and immune response (e.g. IL-6 and IL-1b) (Sen et al., 2007).

Part Two: Methodological Pitfalls in Assessing Relative Contributions of Genes, Environment, and Gene–Environment Interactions in a Disorder with High Heritability

One of the obstacles to a serious consideration of environmental contributors to ASDs has been the high heritability

calculated for this condition, and how heritability has been misinterpreted in some cases. How can environment play a major role in autism if heritability is so high? This is a critical question that arises in discussions of research on environmental risk factors. As explained here, high heritability does not preclude a major role for environmental factors, contrary to common misinterpretations of heritability. Furthermore, there are other ways heritability may be overestimated. For these reasons, population attributable fraction is recommended as a more useful metric for describing the importance of environmental factors in ASDs.

Heritability Is Overestimated Since It Counts GxE as G, and Is Misinterpreted as Ruling out Environment

A heritability estimate describes the percentage of observed phenotypic variance that can be explained by genetic variation in the study. This generally involves taking 100% of the variance in symptoms and splitting it up between genes and environment. Such heritability estimates may be taken to imply that the "impacts" of genes and environment are separable and must sum to 100%.

In particular, twin studies overestimate heritability (and underestimate the role of environment) when genes interact with environmental risk factors that are shared within twin pairs (Nigg, 2006). To the extent a disease is caused by the interaction of genes and shared environment, the importance of shared environment is invisible in such a study design, and all the combined impact of genes and shared environment is misleadingly assigned to genes alone. The direction of bias does depend on whether shared or nonshared environmental effects are involved (Nigg, 2006). Environmental exposures shared by twins may be more common than nonshared exposures during prenatal or perinatal periods, suggesting overestimated heritability is more likely if such exposures are important sources of GxE in ASDs.

Michael Rutter has emphasized the importance of GxE eloquently (2008). GxE may be part of the explanation for the difficulty in finding obvious major causes in either genetics or environment research, despite high heritability estimates.

PAF Is a More Useful Metric for Comparing Interacting Risk Factors

Unfortunately, a heritability estimate of 90% has sometimes been misinterpreted as meaning that only 10% of cases are caused or even influenced by environmental factors. PAF is a more useful metric than heritability if the goal is to describe the proportion of disease burden that could be alleviated through research and interventions focused on a particular risk factor. PAF values estimated for various individual risk factors are not expected to sum to 100%, because the PAF values will overlap where two or more risk factors share responsibility for causation (Ezzati, 2006). Each PAF value accounts for all the cases that might be avoided if the risk factor were removed, even if some of those cases could also be prevented by removal of a different risk factor instead. Autism research would benefit from greater use of PAF estimates.

Heritability May be Overestimated in Other Ways

In addition to GxE, another cause of overestimated heritability may be the impact of shared placentas. Monozygotic (MZ) twins usually share one placenta (*monochorionic, MC*), but sometimes have different placentas (*dichorionic, DC*). Monochorionic twins are more likely to share exposure to certain environmental factors, including pathogens, than dichorionic twins. In this regard it is notable that concordance rates for schizophrenia are higher for monochorionic than for dichorionic twins: "…concordances for MZ twins without MC markers averaged 10.7 percent. In contrast, concordances for MZ twins with one or more MC markers averaged 60 percent." (Davis et al., 1995). This suggests many MZ twins may be concordant because they share a placental environment, not just because they share genes, and heritability may be overestimated.

A heritable change caused by the environment also appears to be a genetic cause, in subsequent generations (once no longer de novo), whether via DNA sequence damage or heritable epigenetic changes. Furthermore, traits influenced by inherited epigenetic modifications appear to be genetic and add to estimates of heritability, when in reality they might result from environmental factors, and could be altered through interventions.

Gene–environment correlation may also affect heritability. It tends to bias heritability estimates upward in the case of nonshared environment, the opposite of how GxE operates. If genes influence a child's behavior and social environment in ways that in turn increase risk of developing an ASD, such environmental factors are again counted as purely genetic and inflate heritability.

Finally, heritability estimates fail to quantify any impacts that lower levels of environmental exposure would have on disease, unless such lower levels are already represented in the study sample. Estimates of the importance of environment in twin studies are limited by the amount of variance in the environment experienced by the study population. Heritability estimates take existing environmental levels and their impacts as a given, and are not designed to measure the baseline impacts of ubiquitous environmental factors.

The Need for Rethinking Heritability

Given the likelihood of GxE, environmentally caused genetic and epigenetic alterations, and other issues raised, it is incumbent upon us to rethink how we have estimated and interpreted heritability, and the roles of genes and environment, in ASDs. PAF could be a valuable metric to use more often in comparisons among risk factors in ASD research.

Part Three: Research Directions and Resources

This chapter has suggested that investigating the combined effects of multiple genes and multiple environmental influences has more potential than studying individual factors in isolation, particularly if the goal is to identify targets for intervention accounting for a larger share of all cases.

Given what is known so far, what next steps might be most productive? Several related approaches are outlined here that may speed advances in autism research, categorized using the framework of four basic causal paths developed in Part One for the ways in which genes and environmental factors may work together in autism. Although a focus on genes within environmentally responsive pathways was introduced and illustrated in Herbert et al. (2006), an expansion is suggested here, to include identifying and studying the combined impacts of both: *(A)* environmental risk factors implicated by autism genetics, and *(B)* genes suggested by environmental risk factors. In other words, genetic findings should inform the focus of environmental research, and vice versa, especially through collaborative studies of combined effects.

(A) Given a candidate autism gene or pathway, identify and study environmental factors that may relate to the key candidate gene or pathway:

1. E→ D: seek environmental factors (e.g., mutagens) that could be a cause of the observed genetic alteration.
2. E→ epiG: seek environmental factors (e.g., nutrition) causing epigenetic changes affecting gene expression (and environment-caused changes in gene expression through other mechanisms).
3. G→ E: seek environmental conditions influenced by the gene (e.g. gaze avoidance, or dietary habits).
4. GxE: seek environmental factors that could interact with the given gene or pathway (GxE), including environmental factors affecting the same pathway already disrupted and made vulnerable by the genetic variant (e.g., pollutants affecting redox status and related metabolic pathways). This approach actually can apply to more than genes or pathways—one might select any alteration found in autism (e.g., alterations in a key brain region, cell type, or biomarker), and then investigate environmental factors reported to affect this parameter. This chapter, however, focuses only on genetic changes, with reported alterations at other levels of biological organization as the starting point for investigating potential environmental factors being beyond the present scope.

(B) Given a candidate environmental factor implicated in autism, identify and study genes and ideally pathways that may relate to the key environmental factor:

1. E→ G: seek genes/pathways most vulnerable to damage by such environmental factors.
2. E→ epiG: seek genes/pathways most vulnerable to epigenetic change resulting from the environmental factor (and genes with altered expression in response to the environmental factor, through any mechanism, not just epigenetic).
3. G→ E: seek genes/pathways that might influence exposure to the environmental factor (e.g., identify any genotypes associated with urban birth or advanced parental age at conception).
4. GxE: seek genes/pathways likely to interact with the environmental factor (GxE), including:
   a. genes in the pathways acutely or permanently disrupted by the environmental factor (e.g., studying acute and permanent gene expression changes that result from the implicated environmental risk factor) or more generally any genes with known roles in some function disrupted by the environmental factor;
   b. genes controlling toxicokinetic parameters (controlling the effective dose of a toxicant resulting from exposure to a given ambient level, through absorption, distribution, metabolism, or elimination of the toxicant and metabolites, such as PON1 or glutathione-related pathways);
   c. genes influencing likelihood of maternal infection or nature of response to infection (e.g., TGFB1);
   d. genes directly affecting sensitivity of genome integrity to environment, including DNA repair genes;
   e. genes affecting genome's sensitivity to epigenetic change caused by environment (e.g., DNA methyltransferases);
   f. genes in stress response pathways in general.

Research opportunities based on the above framework are suggested below. These are followed by some brief references to new informatics resources and research methods that will be helpful in pursuing these opportunities. A conclusion reiterates the importance of shifting from a search for individual genes or single environmental contributors to genomics, combinations of environmental contributors, and systems approaches.

Genetic Implications of Environmental Findings

The environmental factors implicated in autism by studies published to date are likely to be an incomplete and biased list of candidate factors, because a discovery-based approach has not been used to identify potential environmental factors in autism.

A comprehensive, authoritative, standard database summarizing the environmental risk factors implicated in autism does not exist, although this would be a valuable resource. In the absence of such a database, the most comprehensive listing of implicated risk factors may be found in review articles and textbooks Newschaffer et al., 2007; Pessah & Lein, 2008; Lathe, 2006). Environmental risk factors implicated by at least some evidence include maternal infection, urban birth, exposure to certain heavy metals, pesticides, solvents, PCBs, and other

pollutants. Medications and treatments have also been studied as candidate risk factors, including valproic acid and the use of various anticonvulsants for other indications, thalidomide, and assisted reproductive technology. Also implicated are maternal and/or paternal age, which may in turn suggest germ line genetic damage that could partly result from environmental factors such as genotoxins. The few environmental factors implicated to date already provide some leads and suggest the types of genes that may interact with these environmental factors.

The framework discussed above should be applied to environmental findings, as a way to prioritize candidate genes, pathways, and environmental factors for further study. General principles may be useful here as well—a subset of genes can be referred to as *environmentally responsive* genes. These tend to fall in a few key functional categories: cell cycle, DNA repair, cell division, cell signaling, cell structure, gene expression, apoptosis, and metabolism (Herbert et al., 2006; Wilson and Olden, 2004).

Environmental Implications of Genetic and Pathway Findings

In contrast with environmental risk factors, there arguably do exist reasonably comprehensive databases listing the genetic risk factors implicated so far in autism (although no list can claim to include all genomic alterations actually contributing to autism). These genes are one source of information that may suggest candidate environmental risk factors (Corrales, 2009a). Some of the relevant database resources are reviewed in Table 49.2.

Belmonte and Bourgeron (2006) highlight four brain-related features that may contribute to subgroups of ASDs: excess neuronal growth and macrocephaly, abnormal neuromodulatory function, underconnectivity, and overexcitability relative to inhibition (Belmonte & Bourgeron, 2006). From an environmental vantage point, the list would be updated to include oxidative stress, immune system dysregulation, and gender-specific factors, which are environmentally modulated phenomena that may be important in a large percentage of ASD cases, since they are reported in a substantial share of ASD cases but relatively few controls (James et al., 2008; Enstrom et al., 2008). The ASD literature provides many additional clues about potential gene–environment interaction, including findings related to zinc metabolism (Faber et al., 2009; Li et al., 2003), oxytocin (Bartz & Hollander, 2008), steroids (Auyeung et al., 2008; Nakayama et al., 2007), cholesterol synthesis (Tierney et al., 2006; Zecavati & Spence, 2009), circadian rhythms (Melke et al., 2008), and key receptors and pathways including calcium channels and homeostasis (Palmieri et al., 2008; Krey & Dolmetsch, 2007; Pessah, Seegal, et al., 2008). Additionally much can be learned from studying the more extensive research on environmental factors that may influence comorbid disorders (Corrales et al., 2008).

These broad pathways or specific findings in ASDs have implications for the study of environmental risk factors. In general, next steps in pursuing these leads could involve prioritization based on potential for a large population attributable fraction (based on exposure and risk ratio when available), replication in larger samples, and testing for gene–environment interaction in cell-based, animal toxicology, and human epidemiological studies.

Methodologies and Data

In general, biological and environmental databases are multiplying and growing very rapidly. Today, it is far easier to find data on multiple biological molecules or multiple environmental chemicals, and synthesize across databases, than it was just 5 years ago. Table 49.2 reviews some of the resources. Given the paucity of our knowledge, there is a need for discovery-based approaches, which should be applied to screen very broadly, ideally comprehensively, across the full range of genomic/epigenomic factors and environmental factors that might be involved. High-throughput methods and predictive models will be helpful in this regard, and some relevant resources such as the Comparative Toxicogenomics Database (CTD) (Mattingly et al., 2006a, 2006b; Davis et al., 2008) and the Aggregated Computational Toxicology Resource (ACToR) database (Judson et al., 2008) are already useful in compiling available information generated by these methods on gene–toxin interactions. Databases of genes, pathways, and xenobiotics can provide useful tools for network analysis of multiple factors and their potential interactions (see Table 49.2).

Methodological advances are also providing many new opportunities for ASD research. Approaches to identifying gene–environment interactions have been reviewed (Moffitt et al., 2005), and a variety of models are available. Only a very limited number of environmental factors and susceptibilities ave tested so far in such models (Corrales, 2009b), so there is great potential for advances here.

In screening the full range of potential environmental risk factors to determine which ones are worthy of further study, it will be important to consider not only potential toxicity but also the magnitude and timing of exposure. Unfortunately, exposure estimation remains a significant obstacle (Wild, 2005; Cohen-Hubal, 2009), and clearly will require ongoing attention.

Furthermore, the importance of applying a complex systems approach to studying and classifying human diseases is becoming increasingly apparent (Loscalzo et al., 2007; Wu et al., 2008; Sieberts, & Schadt, 2007). Systems biology approaches have been applied to toxicology, and models have been constructed to predict metabolism and toxicity of new chemicals, accounting for genetic variability (Ekins et al., 2006). One important methodological question is what level of detail will be most useful for modeling interactions and causal paths within networks of genes or gene–environment networks. Small to moderately sized networks of genes can be simplified and represented using binary interactions where genes turn other genes on or off, but larger predictive networks, still out of reach with current computational methods,

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Table 49–2.
Selected Informatics Resources Related to Environment and/or Genetics

Database	Location
Exposure and Body Burden Data	
National Health and Nutrition Examination Survey (NHANES) at CDC	http://www.cdc.gov/nchs/nhanes.htm
ExpoCast at EPA (Hubal, 2009)	http://www.epa.gov/ncct/expocast/
Databases at EPA	http://www.epa.gov/epahome/data.html
Gene–Environment Initiative (GEI) at NIH	http://www.gei.nih.gov/exposurebiology/index.asp
ChemIDPlus at NIH ChemIDPlus includes links to databases on household products, food ingredients, medications, illegal drugs, and environmental pollutants.	http://chem.sis.nlm.nih.gov/chemidplus/
Human Microbiome Project at NIH (Turnbaugh et al., 2007)	http://nihroadmap.nih.gov/hmp/
Epidemiology and Toxicology Data	
ACToR and ToxCast databases at EPA's National Center for Computational Toxicology (NCCT) (Judson et al., 2008; Judson et al., 2009; Kavlock et al., 2008)	http://www.epa.gov/comptox/
Office of Environmental Information (OEI), Office of Prevention, Pesticides, and Toxic Substances (OPPTS), and other databases at EPA	http://www.epa.gov/oei/ http://www.epa.gov/oppt/existingchemicals/ http://www.epa.gov/epahome/data.html
Human Genetic Epidemiology (HuGE) Navigator at CDC (including gene–environment interaction literature) (Lin et al., 2006)	http://hugenavigator.net/
Review of NCBI databases at NIH (Sayers et al., 2009)	http://nar.oxfordjournals.org/content/38/suppl_1/D5.abstract
PubChem at NIH (Wang et al., 2009)	http://pubchem.ncbi.nlm.nih.gov/
Comparative Toxicogenomics Database (CTD) The CTD compiles data on and infers relationships between genes, environmental factors, and disorders. (Mattingly, Rosenstein, Colby et al., 2006; Mattingly, Rosenstein, Davis, et al., 2006; Davis et al., 2009)	http://ctd.mdibl.org
NIEHS databases (including links to the Environmental Polymorphism Registry and the Environmental Genome Project, and Chemical Effects in Biological Systems [CEBS] database)	http://www.niehs.nih.gov/research/resources/databases/index.cfm
European eChemPortal	http://www.echemportal.org/
Toxipedia	http://toxipedia.org
Genetic Variant Databases	
Review of top 26 autism gene candidates (Abrahams & Geschwind, 2008)	http://www.ncbi.nlm.nih.gov/pubmed/18414403
Database of genomic variants in autism (Xu et al., 2004; Zhang et al., 2006)	http://projects.tcag.ca/variation/
Autism genetics database (AGD) (includes approximately 226 autism candidate genes as of December 2010) (Matuszek & Talebizadeh, 2009)	http://wren.bcf.ku.edu/
Comparative Toxicogenomics Database (CTD) (includes approximately 220 genes directly related to autism as of December 2010) (Mattingly, Rosenstein, Colby, et al., 2006; Mattingly, Rosenstein, Davis, et al., 2006; Davis, et al., 2009)	http://ctd.mdibl.org/
Autism genetics database (AutDB) (includes approximately 219 autism candidate genes as of December 2010) (Basu et al., 2009)	http://www.mindspec.org/autdb.html
Harvard University project on autism genetics (includes approximately 700 genes with potential relevance to autism as of December 2010) (Wall et al., 2009)	http://autworks.hms.harvard.edu/

(*Continued*)

AU: Please give full term for acronyms at first use

Table 49–2. (Contd.)

Database	Location
Sullivan Lab Evidence Project (SLEP), Psychiatric Genetics database. Compiles data from unbiased genome-wide studies (expression, linkage, association, etc.), so candidate gene studies are not included, nor are studies focused on particular regions of DNA. (Konneker et al., 2008)	http://slep.unc.edu
Genetic Association Database (GAD) at NIH (lists approximately 160 genes studied in autism as of December 2010) (Becker et al., 2004)	http://geneticassociationdb.nih.gov
Human Genetic Epidemiology (HuGE) Gene Prospector at U.S. Centers for Disease Control and Prevention (CDC) (Lists approximately 437 genes that may have been studied in connection with autism as of December 2010). (Yu et al., 2008)	http://hugenavigator.net
Online Mendelian Inheritance in Man (OMIM) at NIH	http://www.ncbi.nlm.nih.gov/omim

will require modeling that clusters genes into functional modules (Bornholdt, 2005).

Systems biology approaches are starting to be applied in the context of ASD research. For example, key signaling pathways are being identified through gene expression analysis of blood-derived lymphoblastoid cells, and are demonstrating that certain key genes or proteins (e.g., excess CYFIP1 and altered levels of JAKMIP1 and GPR155) are consistently dysregulated in autism cases caused by two entirely different causal genetic mutations (FMR1 and 15qdup) (Nishimura et al., 2007). Notably, immune response and mRNA processing were functions impacted by many of the dysregulated genes, and genes implicated in gastrointestinal disease and lipid metabolism were notably overrepresented. Other studies have also analyzed gene expression in ASDs and have implicated immune-related pathways among others (Hu et al., 2006; Gregg et al., 2008; Garbett et al., 2008). Given the influence of environment on so many of these pathways, a GxE approach to further research is strongly indicated.

Conclusion

Future discussions of genetic or environmental risk factors should be motivated by the public and clinical health importance of identifying and prioritizing among all possible contributors, and especially malleable contributors, to ASDs. From this point of view, it is important to make certain that we are turning every stone worth turning, and setting priorities rationally. This chapter has outlined how genetic and environmental factors may converge on common mechanisms pertinent to neurodevelopmental disorders, and the ways that high heritability can involve not only high genetic but also high environmental contributions. This material points toward the strong need for collaboration between genetic and environmental investigators, since this is in the best interests of both. This review has attempted to provide a glimpse of the breadth of substantive arguments for this synergistic approach, and also the many resources that already exist to facilitate it.

Both from an environmental and a medical point of view, ASDs are not only developmental disorders but also chronic conditions. For virtually all major common chronic conditions, finding clear causes, either genetic or environmental, has been difficult, raising fundamental questions about what we are trying to do (Buchanan et al., 2006). Recent systems analyses are finding that not only are there a plethora of interacting causes, but surprisingly there appear to be many core similarities among apparently different diseases (Torkamani et al., 2008). In the face of the prevalence of autism, we should remember as we seek causes that our primary purpose should be to help people with autism; our overriding goal must be to translate our findings into preventing or treating the problems associated with ASDs, and to move eventually to individualized treatments when systems assessments can be integrated into medical practice. At the population level, research should facilitate learning how to provide the greatest improvement in the largest number of cases in the shortest timeframe. This means we need to consider, of the many risk factors, which are most preventable or treatable in the near term and which have the largest impacts (high prevalence of exposure combined with high relative risk results in a high population attributable fraction). There may be important clues gained from rare factors like MECP2, certainly, but their importance at the population level is only in proportion to the extent that they may suggest actionable targets important for most cases of autism.

Michael Rutter argues that our efforts may indeed move faster if we pursue GxE effects:

> "… it is very striking that the G×E effects that have been found are of moderate size and by no means are as small as the main effects of single genes considered independently of the environment. Moreover, the G×E has been found with a sample size of about 1,000. There are a variety of reasons why that is probably rather smaller than is optimal, but it does not necessarily follow that it

is necessary to move to samples of 500,000 as some geneticists have sought to argue." (Rutter, 2008)

Just as there are causes and mechanisms at multiple levels, there will be multiple levels of benefits from going forth and integrating genetic and environmental efforts. The more thorough and rapid generation of results should hasten our ability to provide treatment and prevention.

Challenges and Future Directions

- Misinterpretation of heritability estimates has created the impression that environmental factors play only a minor role in ASDs. Ongoing discussion of gene–environment interaction, mutagens, epigenetics, and gene–environment correlation could help correct any misconceptions.
- Greater collaboration across disciplines would be helpful, particularly between geneticists, toxicologists, and epidemiologists.
- Systematic review and integration across research findings from different disciplines, including PAF calculations, would be helpful in assessing priorities across possible risk factors, biomarkers, and avenues of inquiry.
- Exposure assessment and sample size are substantial obstacles to the study of gene–environment interaction, and deserve ongoing attention. Emerging exposure measurement tools will be extremely helpful, as will large cohort studies such as the National Children's Study.

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