

# Fetal Alcohol Spectrum Disorders: Gene-Environment Interactions, Predictive Biomarkers, and the Relationship Between Structural Alterations in the Brain and Functional Outcomes

James N. Reynolds, PhD,\* Joanne Weinberg, PhD,<sup>†</sup> Sterling Clarren, MD,<sup>‡</sup> Christian Beaulieu, PhD,<sup>§</sup> Carmen Rasmussen, PhD,<sup>||</sup> Michael Kobor, PhD,<sup>¶</sup> Marie-Pierre Dube, PhD,<sup>#</sup> and Daniel Goldowitz, PhD<sup>¶</sup>

Prenatal alcohol exposure is a major, preventable cause of behavioral and cognitive deficits in children. Despite extensive research, a unique neurobehavioral profile for children affected by prenatal alcohol exposure remains elusive. A fundamental question that must be addressed is how genetic and environmental factors interact with gestational alcohol exposure to produce neurobehavioral and neurobiological deficits in children. The core objectives of the NeuroDevNet team in fetal alcohol spectrum disorders is to create an integrated research program of basic and clinical investigations that will (1) identify genetic and epigenetic modifications that may be predictive of the neurobehavioral and neurobiological dysfunctions in offspring induced by gestational alcohol exposure and (2) determine the relationship between structural alterations in the brain induced by gestational alcohol exposure and functional outcomes in offspring. The overarching hypothesis to be tested is that neurobehavioral and neurobiological dysfunctions induced by gestational alcohol exposure are correlated with the genetic background of the affected child and/or epigenetic modifications in gene expression. The identification of genetic and/or epigenetic markers that are predictive of the severity of behavioral and cognitive deficits in children affected by gestational alcohol exposure will have a profound impact on our ability to identify children at risk.

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From the \*Department of Pharmacology and Toxicology, Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada.

<sup>†</sup>Department of Cellular and Physiological Sciences, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada.

<sup>‡</sup>Centre for Community Child Health Research, L408 Canada Northwest FASD Research Network, Vancouver, British Columbia, Canada.

<sup>§</sup>Department of Biomedical Engineering, University of Alberta, Edmonton, Alberta, Canada.

<sup>||</sup>Section of Pediatric Neurosciences, Department of Pediatrics, Glenrose Rehabilitation Hospital, University of Alberta, Edmonton, Alberta, Canada.

<sup>¶</sup>Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada.

<sup>#</sup>Department of Medicine, Université de Montréal and Statistical Genetics Research Group, Montreal Heart Institute, Montreal, Quebec, Canada.

Address reprint requests to James N. Reynolds, PhD, Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, K7L 3N6. E-mail: jnr@queensu.ca

The term fetal alcohol syndrome (FAS) was introduced over 30 years ago as a diagnosis for children who exhibit the classic triad of central nervous system dysfunction, growth deficiency, and characteristic craniofacial dysmorphism resulting from maternal consumption of excessive amounts of alcohol during pregnancy. The most debilitating aspect of prenatal alcohol exposure is central nervous system injury, which can manifest as intellectual, neurologic, and behavioral abnormalities. Although FAS is believed to occur in approximately 1 to 3 per 1,000 live births in North America, gestational alcohol exposure at levels lower than those that result in the full syndrome produce a wider range of neurobiological and neurobehavioral abnormalities. The full spectrum of adverse effects, which includes several diagnostic subgroups, is collectively referred to as fetal alcohol spectrum disorders (FASDs). Recently published estimates suggest that FASD may occur as frequently as 2 to 5 per 1,000 live births,<sup>1</sup> making this a public health problem of epidemic proportion.

The brain is the principal target organ for alcohol teratogenesis, and the brain injury of FASD is by far the most common and serious part of the condition. Animal experimentation has shown that alcohol more often produces neurochemical and structural changes throughout the brain rather than gross malformations. In the human, these changes may go undetected for many years until the child reaches an age when normal functions should be maturing but are impaired. The consequences of prenatal alcohol exposure can then manifest in a wide variety of mild to moderate brain dysfunctions in processes, such as learning and memory, executive function, social communication, attention, and sensory-motor skills. Childhood depression, anxiety, and other mental health conditions are also common and might be either manifestations of the primary brain alterations or may be secondary to the neurobehavioral/neurobiological alterations that occur. The combination of these functional deficits leads to severe adaptive problems at home, at school or work, and in society. A recent study that examined key cost components (direct costs: medical, education, social services, and out-of-pocket costs and indirect costs: productivity losses) estimated that the total adjusted annual cost associated with FASD in Canada is \$5.3 billion.<sup>2</sup>

Although many neurobehavioral deficits have been described, a unique neurobehavioral profile for FASD remains elusive. Importantly, the sometimes wide variability in the phenotypic presentation of children affected by gestational alcohol exposure has led to the realization that alcohol-induced teratogenic outcomes are most likely determined by a combination of genetic and epigenetic modifications interacting with environmental influences. For example, in a study involving 16 alcohol-exposed twin pairs,<sup>3</sup> concordance of diagnosis was reported for 5 of 5 monozygotic twin pairs, but discordance of diagnosis was reported for 7 of 11 dizygotic twin pairs. Animal models have clearly established that the genetic background of the mother has a major influence on ethanol teratogenesis but that maternal-fetal interactive effects also play a significant role.<sup>4</sup>

Polymorphisms in alcohol metabolizing enzymes have been suggested to have a significant impact on the risk for FASD. The primary enzyme responsible for ethanol metabolism, alcohol dehydrogenase (ADH), exists as 3 distinct isozymes encoded by 3 members of the ADH family: *ADH1A*, *ADH1B*, and *ADH1C*. Moreover, 3 functionally important polymorphisms exist for *ADH1B* and 2 for *ADH1C*, which affect the binding affinity for substrate and the maximum rate of alcohol metabolism. For example, variations in the alcohol dehydrogenase gene (*ADH1B*) have been reported to confer either increased or decreased likelihood of developing FASD.<sup>5-8</sup> Clearly, more studies are needed to clarify the role of variability in alcohol metabolism on the occurrence of FASD and to test the contribution of other gene products. FASD is a complex and multifactorial set of disorders, and many other genes are likely to play critical roles in the susceptibility to ethanol teratogenesis. Thus, a fundamental issue in the search for effective therapeutic interventions in FASD is to understand how genetic and environmental fac-

tors interact with gestational alcohol exposure to produce neurobehavioral and neurobiological deficits in children.

Epigenetics refers to genome information not encoded in the DNA sequence, with the best understood consequence of epigenetic marks being the control of gene expression. Epigenetic mechanisms that influence gene expression include DNA methylation and/or changes in chromatin structure and are vital in controlling how genes interact with the environment.<sup>9</sup> DNA methylation is a very stable and accessible epigenetic mark and, therefore, the most suitable for population-based epigenetic studies. Given the extensive crosstalk between different chromatin marks, DNA methylation can also serve as a marker for general alterations of epigenetic modifications at a given gene promoter.

Investigation of possible epigenetic mechanisms as mediators of alcohol's adverse effects on the fetus provides another promising approach for understanding the FASD phenotype.<sup>10,11</sup> Epigenetic mechanisms refer to changes in genome information caused by stable but possibly reversible alterations that do not involve changes in the underlying DNA sequence. Because of the dynamic nature of the interaction, epigenetic mechanisms provide an attractive concept as mediators connecting the genome to environmental signals and exposure. The overarching hypothesis to be tested by the NeuroDevNet team in FASD is that neurobehavioral and neurobiological dysfunctions induced by gestational alcohol exposure are correlated to the genetic background of the affected child and/or epigenetic modifications in gene expression.

Studies conducted by members of the FASD research team have investigated deficits in executive function,<sup>12-14</sup> structural brain imaging,<sup>15,16</sup> and saccadic eye movements<sup>17</sup> in children with FASD. The program of studies to be performed by NeuroDevNet will use these novel methodologies in a much larger cohort of children by undertaking a multisite study across Canada. Coupled with extensive neurobehavioral testing and genetic/epigenetic analyses, a powerful and unique dataset will be generated that can be mined for interactions between environmental and genomic factors that contribute to adverse outcomes in children affected by prenatal alcohol exposure.

The core objectives of the NeuroDevNet Team in FASD is to create an integrated research program of basic and clinical investigations that will (1) identify genetic and epigenetic modifications that may be predictive of the neurobehavioral and neurobiological dysfunctions induced by gestational alcohol exposure (these would be potential biomarkers of risk and thus would be a major advance in the field) and (2) determine the relationship between structural alterations in the brain induced by gestational alcohol exposure and functional outcomes or genetic groupings in children with FASD relative to control subjects.

## Research Plan

### Clinical Studies

Each FASD diagnostic clinic within the network that agrees to participate in the research program will identify male and

female children ages 5 to 18 years with confirmed gestational alcohol exposure. Willing participants will complete a standardized battery of psychometric tests that will assess multiple central nervous system domains (eg, executive function, working memory, and language and math skills). A similar-sized age- and sex-matched control group of typically developing children will be recruited from the same geographic regions and will also be given the same standardized tests. Buccal swabs and saliva samples will be taken for the examination of physiological, genetic, and epigenetic markers; novel neurobiological testing involving saccadic eye movements will be performed; and refined and expanded brain-imaging analysis will be performed.

### Physiological analysis

The hypothalamic-pituitary-adrenal (HPA) axis is known to be highly susceptible to programming during fetal and neonatal development.<sup>18</sup> Data indicate that prenatal exposure to ethanol is an early environmental insult that can program the offspring HPA axis such that HPA tone is increased throughout life, as reflected by higher basal and poststress cortisol concentrations in 13-month-old infants.<sup>19</sup> Similarly, Haley et al<sup>20</sup> reported that prenatal alcohol exposure was associated with greater cortisol reactivity, a negative affect, and an elevated heart rate. Of relevance, these effects differed in boys and girls, with girls showing greater changes in heart rate and negative affect than boys and boys showing greater changes in cortisol than girls. Altered HPA development and regulation is thought to be a risk marker for cognitive deficits<sup>21</sup> and socioemotional adjustment.<sup>22</sup> Members of our team have recently shown that the cortisol awakening response may serve as a useful index of adrenocortical reactivity in young children, possibly signaling a disturbance in physiological regulation.<sup>23</sup> In the current study, we will examine the cortisol circadian rhythm as a measure of basal HPA regulation and reactivity by collecting and assaying saliva samples collected from children in the morning (cortisol awakening response) and at bedtime.

### Genetic Analysis

In the candidate gene approach, the genetic determinants of risk for FASD can best be studied using a candidate-gene strategy based on the existing knowledge of biological mechanisms of action. This approach has the advantage of focusing resources on a manageable number of gene mutations and polymorphisms that are likely to be important. Candidate gene-association studies remain the most practical approach to complex, multifactorial disorders, such as FASD, which are most likely characterized by relatively weak genotype-phenotype associations.

For single nucleotide polymorphism (SNP) identification and selection, the selection of SNPs for genotyping will be prioritized based on published literature implicating functional relevance in FASD. An initial list of 90 to 100 genes mined from published literature<sup>24</sup> will serve as the starting point. SNPs with haplotype tagging potential will also be favored, and we focus on SNPs with reasonable allele fre-

quency ( $\geq 30\%$ ) in the population. Regulatory SNPs will be targeted by using bioinformatics tools that predict promoter elements in genomic sequence as well as areas of strong cross-species conservation.

After the finding of a positive or borderline association with candidate gene SNPs, selected patient samples will be chosen for sequencing to identify the putative causal variant in the associated genomic regions. Sequencing will be directed to the open reading frame as well as to conserved potentially regulatory genomic regions. This will allow us to detect any additional polymorphisms that would be informative for follow-up association testing in the full patient population. We will be able to search an associated region for causal polymorphisms and ultimately to follow-up the findings by molecular validation. The primary objective of the FASD candidate-gene analysis will be the identification of genetic determinants that may be predictive of the severity of brain dysmorphology and dysfunction in children with FASD.

### Epigenetic Analysis

The aim of this component of the study is to decipher the circuitry between the genome, the epigenome, and the environment, specifically as it relates to the long-term regulation of transcriptional programs associated with prenatal alcohol exposure. The integration of epigenome data from human subjects and the rodent models (see later) is central to the strategy used and will allow for the identification of salient changes relevant to brain development in the etiology of FASD.

Using buccal cells obtained from children enrolled in this study, we examine fluctuations in DNA methylation, with the aim of distinguishing signature epigenome profiles in children with FASD compared with children in our control sample. Such a signature might not only be helpful in understanding the molecular etiology of FASD but also might serve as a tool for early perinatal diagnosis of FASD. DNA methylation will be measured by a mixture of unbiased array-based approaches and more extensive quantitative measurements of candidate gene promoter regions. The latter will include a specific panel of genes involved in regulating the HPA axis and general stress response, genes involved in neuropeptides hormone activity and key neurotransmitter systems (eg, serotonin and dopamine), and genes, such as NR2B and  $\alpha$ -synuclein, whose DNA methylation is altered in subjects with alcohol dependency. Moreover, we will examine genes involved in protein synthesis, turnover and folding, cellular metabolism, and signal transduction pathways, all of which are critical during development.<sup>25,26</sup> To examine the potential circuitry between genetic variation and epigenetic marks, we also include in this panel promoter regions of genes that might predispose to FASD, drawn from the set interrogated in the genotype analysis.

### Imaging Studies

Three main brain abnormalities that are commonly associated with FASD are small head (and brain) relative to body size, thin corpus callosum, and underdeveloped cerebellum.<sup>27-29</sup> However, these brain abnormalities are insufficient

to explain the range of deficits observed in these children. Indeed, conventional magnetic resonance imaging (MRI) is often interpreted clinically as unremarkable or nonspecific, which implies that standard imaging techniques are insensitive. Quantitative imaging has been more sensitive at documenting the brain regions that may be most susceptible to gestational alcohol exposure.<sup>30</sup> Volumetric assessment of 3-dimensional T1-weighted MRI scans have shown that white-matter volume appears to be disproportionately reduced in individuals with prenatal alcohol exposure<sup>31,32</sup> with alterations in the corpus callosum (the largest white-matter tract that connects the 2 cerebral hemispheres) as the most frequently reported findings.<sup>33</sup> Deep gray-matter structures, such as the basal ganglia, have exhibited volume reductions.<sup>34,35</sup> Studies of the hippocampus, which is important for memory, have been more variable; 1 study reported asymmetry in subjects with FASD,<sup>36</sup> whereas another suggested that the hippocampus was spared.<sup>31</sup> Imaging studies have also shown abnormalities in both brain function<sup>35,37</sup> and metabolism.<sup>38,39</sup> Cortical thickness was greater in the bilateral temporal, bilateral inferior parietal, and right frontal regions of subjects with heavy prenatal exposure, perhaps reflecting either reduced cortical thinning as expected in healthy development or less cortical infiltrating myelination from adjacent white matter.<sup>40</sup> The cingulate gyrus, both white and gray matter, has also been shown to be reduced in youths with heavy prenatal alcohol exposure.<sup>41</sup> Because there has been only 1 study on regional cortical thickness using advanced processing tools in children with FASD and a handful of inconsistent studies on the deep brain structures, such as the hippocampus (most likely a consequence of small sample size), these 2 areas warrant further investigation.

White matter may be more sensitive to alcohol-induced brain injury in the fetus because glial cells, which both guide the structural development of the brain and myelinate axons, appear to be particularly vulnerable to alcohol toxicity.<sup>42</sup> An excellent method of measuring the white matter is diffusion tensor magnetic resonance imaging (DTI),<sup>43</sup> which permits the virtual dissection of specific white-matter tracts (ie, the wiring) in the living brain<sup>44-47</sup> and provides quantitative parameters, such as fractional anisotropy (FA) and mean diffusivity that can be compared between subjects.<sup>48</sup> DTI may provide a more sensitive measure of tissue microstructure than conventional MRI. Mean diffusivity is a measure of the bulk water diffusion, whereas FA measures the degree of water diffusion directionality, an indirect measure of structural integrity (eg, linked to axonal packing and degree of myelination).<sup>49</sup> DTI studies of FASD in children and young adults have shown diffusion abnormalities in the corpus callosum.<sup>50-53</sup> However, given the variety of neurologic and cognitive deficits associated with FASD, other brain regions are likely to be affected and warrant investigation. A whole-brain, voxel-based analysis of children and adolescents with FASD showed anisotropy reductions in the splenium of the corpus callosum and right lateral temporal lobe.<sup>54</sup> In a study conducted by FASD team members of children with FASD,<sup>15</sup> tractography and region of interest analysis revealed much more widespread diffusion abnormalities than all earlier

studies, namely, in 7 of 10 white-matter tracts and 3 of 4 deep gray-matter structures. Children with FASD showed a decrease in total brain volume relative to controls with a greater percent reduction of white matter than gray matter. Children with FASD also showed below-average performance on neuropsychological tests, with particular deficits in working memory, quantitative concepts, vocabulary, and executive functioning measures; however, the diffusion parameters averaged over entire tracts did not correlate with any of the cognitive tests in this small group.<sup>15</sup> When a voxel-by-voxel-based analysis was used, correlations between math ability and white-matter FA were found in several regions, notably the intraparietal sulcus.<sup>16</sup> Nonetheless, a larger cohort of children with FASD is needed to establish the exact relationship between structural alterations in the brain and neurobehavioral and cognitive deficits. Furthermore, there are no studies relating specific brain abnormalities observed using MRI with epigenetic traits; this is important because it is presumed that not all individuals are affected the same by similar maternal alcohol use.

## Saccadic Eye Movements

The measurement of eye movement control is a powerful tool for assessing sensory, motor, and cognitive function. Saccades are rapid eye movements that bring new visual targets onto the fovea of the retina (the region of highest visual acuity). They can be generated volitionally or automatically in response to sensory stimuli that appear suddenly. The specific regions of the brain responsible for the voluntary control of saccades such as the frontal cortex, basal ganglia, and brainstem centers are well understood.<sup>55</sup>

Studies conducted in our laboratories and by others have shown that deficits in eye movement control can be measured with a high degree of sensitivity in children with neurodevelopmental disorders, including attention-deficit hyperactivity disorder, FASD, and autism spectrum disorders.<sup>17,56-58</sup> Our team has developed approaches to measure eye movements in both structured and unstructured tasks (eg, watching video clips). Recent technological advances for the tracking and recording of eye movements use remote camera assemblies that allow for substantial movement of the subject's head without the loss of the eye-tracking signal. These setups allow for more natural, free-viewing sessions in which the subject performs eye movement tasks or simply watches video clips projected onto a liquid crystal display monitor. Saccadic eye movement data are collected automatically, and the data are used to quantify performance deficits and to create behavioral patterns based on the salience model of Itti and Koch.<sup>59</sup> The primary hypothesis driving this project is that there is sufficient predictive information in the performance of eye movement tasks to objectively identify meaningful groupings of children with FASD.

## Animal Studies

### Mouse

The study of FASD genetics is greatly hampered by multiple issues, the prime one of which is that exposure levels are intrinsically variable and the maternal influence is typically



difficult to assess. Here, the use of mouse models is particularly valuable in terms of being able to use standardized gestational timing of exposure and dose of ethanol and for assessing the maternal component. Furthermore, animal models, particularly rodent models, of FASD have been well explored and represent viable and developmentally relevant approaches to understanding various aspects of FASD.

It is well established that the C57BL/6J strain of mice is very sensitive, whereas the DBA/2J strain is relatively resistant to ethanol teratogenesis.<sup>4</sup> From these parental strains, a set of recombinant inbred mouse lines have been created by inbreeding progeny from a C57BL/6J X DBA/2J F2 intercross.<sup>60</sup> This set of mice (termed BXD recombinant inbred lines), which has recently been expanded to include approximately 80 lines, has become a powerful tool for studies of complex trait genetics. This is because the genetic variation in BXD mice is between lines rather than between animals, and, therefore, the BXD panel serves as a genetic reference population. With the expansion of this reference panel to over 80 lines, it represents the largest cohort and provides the most power in identifying the genetic architecture of complex traits.

The BXD panel of mice has been shown to have the capability to detect quantitative trait loci for a variety of traits related to brain function.<sup>61</sup> The strength and simplicity of this approach is that each of the lines has been extensively genotyped, and, therefore, the work that needs to go into the phenotype-to-genotype correlation is simply obtaining the phenotypic read out. Members of the FASD team have shown that extensive cell death occurs in widespread regions of the C57BL/6J brain after both prenatal and postnatal ethanol administration, whereas the DBA/2J brain exhibits attenuated cell death. Using experimental and bioinformatics approaches, we will test the hypothesis that specific genes are associated with ethanol teratogenesis.

## Rat

The ability to ask parallel questions and to obtain parallel measures in human and animal studies is a critical strength of this network. In the rat model, as in the mouse model described previously, one has unique control of conditions not possible in human studies, including the dose and timing of alcohol exposure, maternal variables, genetic variables, and the pre- versus postnatal environment.<sup>62</sup> In addition, one can obtain parallel measures of epigenetic markers in buccal epithelium, blood, and brain to determine how closely measures in peripheral compartments reflect central changes.

Animals exposed to alcohol prenatally will be tested in adulthood on a broad battery of behavioral tests focusing on tasks that reflect primarily cognitive and executive (prefrontal cortex) functions, domains known to be altered by prenatal exposure to alcohol and parallel, in many respects, domains targeted in the neuropsychological testing to be carried out in the children.

To link the animals and clinical studies, blood samples, buccal swabs, and brains will be collected from all experimental groups for analyses of gene-expression profiles and

epigenetic markers. The power of this design is that we can directly test the hypothesis that changes in gene expression detected in blood lymphocytes (the typical source in many human studies) and buccal epithelium (the human source in the studies in this proposal) are related to changes occurring in key brain structures believed to be especially sensitive to the teratogenic effects of prenatal alcohol exposure. Brain areas to be dissected for these initial studies will include the hippocampus, hypothalamus, amygdala, and prefrontal cortex.

## Anticipated Impact and Significance

The approaches undertaken in the NeuroDevNet FASD project will allow us to build the translational link between the animal data and the human studies, which will provide the basis for improved identification and diagnosis of FASD and for the development of targeted interventions, including novel therapies. These studies reflect the strong integration of basic and clinical investigations that form the foundation of NeuroDevNet. The identification of genetic and epigenetic markers that are predictive of the severity of behavioral and cognitive deficits in children affected by gestational alcohol exposure will have a profound impact on our ability to identify children at risk. Early and accurate diagnosis of a child with FASD remains a substantive clinical challenge, and there is a clear need to develop new approaches that can be used to mitigate the brain injury on a population basis. Established animal models will be used to confirm the role of candidate genes suggested from the human studies and will be used to identify markers in buccal epithelial cells that are reflective of alterations in gene expression in the brain.

NeuroDevNet will generate new knowledge that will form the basis for evidence-based treatments to mitigate the neurobehavioral and neurobiological disturbances in FASD and, thus, the life-time burden on the individual, family, and society. A key deliverable of NeuroDevNet will be knowledge translation to clinicians and other health care professionals concerning the potential impact of early therapeutic interventions on long-term outcome for children affected by FASD. These interventions will ultimately be evaluated over the long-term to assess their impact on the health of each individual child and, thus, the direct and indirect cost savings associated with early diagnosis of FASD can be determined for Canada.

## References

1. May PA, Gossage JP, Kalberg WO, et al: Prevalence and epidemiological characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Dev Disabil Res Rev* 15:176-192, 2009
2. Stade B, Ali A, Bennett D, et al: The burden of prenatal exposure to alcohol: Revised measurement of cost. *Can J Clin Pharmacol* 16:e91-102, 2009
3. Streisguth AP, Dehaene P: Fetal alcohol syndrome in twins of alcoholic mothers: Concordance of diagnosis and IQ. *Am J Med Genet* 47:857-861, 1993

4. Warren KR, Li T-K: Genetic polymorphisms: Impact on the risk of fetal alcohol spectrum disorders. *Birth Defects Res A Clin Mol Teratol* 73: 195-203, 2005
5. Jacobson SW, Carr LG, Croxford J, et al: Protective effects of the alcohol dehydrogenase-ADH1B allele in children exposed to alcohol during pregnancy. *J Pediatr* 148:30-37, 2006
6. McCarver DG, Thomasson HR, Martier SS, et al: Alcohol dehydrogenase-2\*3 allele protects against alcohol-related birth defects among African Americans. *J Pharmacol Exp Ther* 283:1095-1101, 1997
7. Stoler JM, Ryan LM, Holmes LB: Alcohol dehydrogenase 2 genotypes, maternal alcohol use, and infant outcome. *J Pediatr* 141:780-785, 2002
8. Viljoen DL, Carr LG, Foroud TM, et al: Alcohol dehydrogenase-2\*2 allele is associated with decreased prevalence of fetal alcohol syndrome in the mixed-ancestry population of the Western Cape Province, South Africa. *Alcohol Clin Exp Res* 25:1719-1722, 2001
9. Henikoff S, Matzke MA: Exploring and explaining epigenetic effects. *Trends Genet* 13:293-295, 1997
10. Haycock PC: Fetal alcohol spectrum disorders: The epigenetic perspective. *Biol Reprod* 81:607-617, 2009
11. Haycock PC, Ramsay M: Exposure of mouse embryos to ethanol during preimplantation development: Effect on DNA methylation in the H19 imprinting control region. *Biol Reprod* 81:618-627, 2009
12. Rasmussen C: Executive functioning and working memory in fetal alcohol spectrum disorder. *Alcohol Clin Exp Res* 29:1359-1367, 2005
13. Rasmussen C, Bisanz J: Executive functioning in children with Fetal Alcohol Spectrum Disorders: Profiles and age-related differences. *Child Neuropsychol* 15:201-215, 2009
14. Green CR, Mihic AM, Nikkel SM, et al: Executive function deficits in children with fetal alcohol spectrum disorders (FASD) measured using the Cambridge Neuropsychological Tests Automated Battery (CANTAB). *J Child Psychol Psychiatry* 50:688-697, 2009
15. Lebel C, Rasmussen C, Wyper K, et al: Brain Diffusion Abnormalities in children with fetal alcohol spectrum disorder. *Alcohol Clin Exp Res* 32:1732-1740, 2008
16. Lebel C, Rasmussen C, Wyper K, et al: Brain microstructure is related to math ability in children with fetal alcohol spectrum disorder. *Alcohol Clin Exp Res* 34:354-363, 2010
17. Green CR, Mihic AM, Brien DC, et al: Oculomotor deficits in children with fetal alcohol spectrum disorders assessed using a mobile laboratory. *Eur J Neurosci* 29:1302-1309, 2009
18. Welberg LA, Seckl JR: Prenatal stress, glucocorticoids and the programming of the brain. *J Neuroendocrinol* 13:113-128, 2001
19. Jacobson SW, Bihun JT, Chiodo LM: Effects of prenatal alcohol and cocaine exposure on infant cortisol levels. *Dev Psychopathol* 11:195-208, 1999
20. Haley DW, Handmaker NS, Lowe J: Infant stress reactivity and prenatal alcohol exposure. *Alcohol Clin Exp Res* 30:2055-2064, 2006
21. Lupien S, Lecours AR, Lussier I, et al: Basal cortisol levels and cognitive deficits in human aging. *J Neurosci* 14:2893-2903, 1994
22. Smider NA, Essex MJ, Kalin NH, et al: Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: A prospective study. *Child Dev* 73:75-92, 2002
23. Scher A, Hall WA, Zaidman-Zait A, et al: Sleep quality, cortisol levels, and behavioral regulation in toddlers. *Dev Psychobiol* 52:44-53, 2010
24. Lombard Z, Tiffin N, Hofmann O, et al: Computational selection and prioritization of candidate genes for fetal alcohol syndrome. *BMC Genomics* 8:389-404, 2007
25. Stead JD, Neal C, Meng F, et al: Transcriptional profiling of the developing rat brain reveals that the most dramatic regional differentiation in gene expression occurs postpartum. *J Neurosci* 26:345-353, 2006
26. Weaver ICG, Meaney MJ, Szyf M: Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci U S A* 103:3480-3485, 2006
27. Chudley AE, Conry J, Cook JL, et al: Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. *CMAJ* 172:S1-S21, 2005
28. Astley SJ, Aylward EH, Olson HC, et al: Magnetic resonance imaging outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* 33:1671-1689, 2009
29. Astley SJ, Richards T, Aylward EH, et al: Magnetic resonance spectroscopy outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. *Magn Reson Imaging* 27:760-778, 2009
30. Norman AL, Crocker N, Mattson SN, et al: Neuroimaging and fetal alcohol spectrum disorders. *Dev Disabil Res Rev* 15:209-217, 2009
31. Archibald SL, Fennema-Notestine C, Gamst A, et al: Brain dysmorphology in individuals with severe prenatal alcohol exposure. *Dev Med Child Neurol* 43:148-154, 2001
32. Sowell ER, Thompson PM, Mattson SN, et al: Voxel-based morphometric analyses of the brain in children and adolescents prenatally exposed to alcohol. *Neuroreport* 12:515-523, 2001
33. Riley EP, Mattson SN, Sowell ER, et al: Abnormalities of the corpus callosum in children prenatally exposed to alcohol. *Alcohol Clin Exp Res* 19:1198-1202, 1995
34. McGee CL, Riley EP: Brain imaging and fetal alcohol spectrum disorders. *Ann Ist Super Sanita* 42:46-52, 2006
35. Spadoni AD, McGee CL, Fryer SL, et al: Neuroimaging and fetal alcohol spectrum disorders. *Neurosci Biobehav Rev* 31:239-245, 2007
36. Riikonen R, Salonen I, Partanen K, et al: Brain perfusion SPECT and MRI in foetal alcohol syndrome. *Dev Med Child Neurol* 41:652-659, 1999
37. Sowell ER, Lu LH, O'Hare ED, et al: Functional magnetic resonance imaging of verbal learning in children with heavy prenatal alcohol exposure. *Neuroreport* 18:635-639, 2007
38. Cortese BM, Moore GJ, Bailey BA, et al: Magnetic resonance and spectroscopic imaging in prenatal alcohol-exposed children: Preliminary findings in the caudate nucleus. *Neurotoxicol Teratol* 28:597-606, 2006
39. Fagerlund A, Heikkinen S, Autti-Ramo I, et al: Brain metabolic alterations in adolescents and young adults with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* 30:2097-2104, 2006
40. Sowell ER, Mattson SN, Kan E, et al: Abnormal cortical thickness and brain-behavior correlation patterns in individuals with heavy prenatal alcohol exposure. *Cereb Cortex* 18:136-144, 2008
41. Bjorkquist OA, Fryer SL, Reiss AL, et al: Cingulate gyrus morphology in children and adolescents with fetal alcohol spectrum disorders. *Psychiatry Res* 181:101-107, 2010
42. Guerri C, Pascual M, Renau-Piqueras J: Glia and fetal alcohol syndrome. *Neurotoxicology* 22:593-599, 2001
43. Basser P, Mattiello J, Le Bihan D: Estimation of the effective self-diffusion tensor from the NMR spin echo. *J Magn Reson B* 103:247-254, 1994
44. Basser PJ, Pajevic S, Pierpaoli C, et al: In vivo fiber tractography using DT-MRI data. *Magn Reson Med* 44:625-632, 2000
45. Conturo TE, Lori NF, Cull TS, et al: Tracking neuronal fiber pathways in the living human brain. *Proc Natl Acad Sci U S A* 96:10422-10427, 1999
46. Jones DK, Simmons A, Williams SC, et al: Non-invasive assessment of axonal fiber connectivity in the human brain via diffusion tensor MRI. *Magn Reson Med* 42:37-41, 1999
47. Mori S, Crain BJ, Chacko VP, et al: Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Ann Neurol* 45:265-269, 1999
48. Le Bihan D: Looking into the functional architecture of the brain with diffusion MRI. *Nat Rev Neurosci* 4:469-480, 2003
49. Beaulieu C: The basis of anisotropic water diffusion in the nervous system—A technical review. *NMR Biomed* 15:435-455, 2002
50. Ma X, Coles CD, Lynch ME, et al: Evaluation of corpus callosum anisotropy in young adults with fetal alcohol syndrome according to diffusion tensor imaging. *Alcohol Clin Exp Res* 29:1214-1222, 2005
51. Wozniak JR, Mueller BA, Chang PN, et al: Diffusion tensor imaging in children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* 30:1799-1806, 2006
52. Wozniak JR, Muetzel RL, Mueller BA, et al: Microstructural corpus callosum anomalies in children with prenatal alcohol exposure: An

- extension of previous diffusion tensor imaging findings. *Alcohol Clin Exp Res* 33:1825-1835, 2009
53. Li L, Coles CD, Lynch ME, et al: Voxel wise and skeleton-based region of interest analysis of fetal alcohol syndrome and fetal alcohol spectrum disorders in young adults. *Hum Brain Map* 30:3265-3274, 2009
54. Sowell ER, Johnson A, Kan E, et al: Mapping white matter integrity and neurobehavioral correlates in children with fetal alcohol spectrum disorders. *J Neurosci* 28:1313-1319, 2008
55. Munoz DP, Everling S: Look away: The antisaccade task and the voluntary control of eye movement. *Nat Rev Neurosci* 5:218-228, 2004
56. Green CR, Nikkel S, Munoz DP, et al: Deficits in eye movement control in children with Fetal Alcohol Spectrum Disorders. *Alcohol Clin Exp Res* 31:500-511, 2007
57. Mostofsky SH, Lasker AG, Singer HS, et al: Oculomotor abnormalities in boys with Tourette syndrome with and without ADHD. *J Am Acad Child Adolesc Psychiatry* 40:1464-1472, 2001
58. Munoz DP, Armstrong IT, Hampton KA, et al: Altered control of visual fixation and saccadic eye movements in attention-deficit hyperactivity disorder. *J Neurophysiol* 90:503-514, 2003
59. Itti L, Koch C: Computational modeling of visual attention. *Nat Rev Neurosci* 2:194-203, 2001
60. Peirce JL, Lu L, Gu J, et al: A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet* 5:7, 2004
61. Philip VM, Duvurru S, Gomero B, et al: High-throughput behavioral phenotyping in the expanded panel of BXD recombinant inbred strains. *Genes Brain Behav* 9:129-159, 2010
62. Hellemans KG, Sliwowska JH, Verma P, et al: Prenatal alcohol exposure: Fetal programming and later life vulnerability to stress, depression and anxiety disorders. *Neurosci Biobehav Rev* 34:791-807, 2010