Ethical considerations for biomarkers of fetal alcohol spectrum disorder and other neurodevelopmental disorders

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Developments in Neuroethics and Bioethics
ISSN 2589-2959
https://doi.org/10.1016/bs.dnb.2023.05.003

1. Introduction

The development and implementation of biomarkers that are both sensitive and specific to different disorders is a long-standing goal of health research. Indeed, many success stories in human health are the result of these efforts; for instance, phenylketonuria (PKU), an inherited disorder that leads to intellectual disability and other serious health problems when untreated, is now identified immediately at birth thanks to the Guthrie test. By identifying affected children at birth, clinicians are now positioned to provide treatments that can prevent the deleterious effects of PKU. Other success stories include biomarkers and diagnostic tests for cancer, cardiovascular disease, diabetes, etc., which are crucial to effective early treatments for these disorders at the individual and population level. By contrast, the vast majority of neurodevelopmental disorders do not yet have effective biomarkers. Neurodevelopmental disorders can present through a wide range of symptoms and phenotypes, many of which may overlap to some extent with each other and can be associated with considerable stigma and shame related to their diagnosis. The similarities that can occur among neurodevelopmental disorders further highlight the difficulties in differentiating among disorders in both clinical and research settings, which can have profound impacts on diagnosis and subsequent treatment. In addition to overlapping symptoms, many of these disorders can be co-occurring, leading to additional variance and difficulties in the management of symptoms and treatments. As such, it is crucial for clinicians and researchers alike to differentiate among these disorders and provide accurate diagnoses or characterizations of the different neurodevelopmental disorders. In the present chapter, we use fetal alcohol spectrum disorder (FASD) as an exemplar to demonstrate the importance of biomarkers for neurodevelopmental disorders and emphasize the ethical considerations surrounding their application in research and clinical settings.

FASD refers to the broad range of effects that can occur in an individual with prenatal alcohol exposure, which include physical, behavioral, neurodevelopmental, and/or learning disabilities that can last a lifetime (Bertrand, Floyd, & Weber, 2005). A variety of diagnostic guidelines have been developed over the years to assist clinicians in recognizing and diagnosing children exposed to alcohol in utero (Benz, Rasmussen, & Andrew, 2009). While many guidelines require the presence of physical characteristics, including facial dysmorphology and other birth defects that can result from prenatal

exposure to alcohol, for at least some diagnoses within the spectrum, data from clinical studies suggest that only about 10% of individuals exposed to alcohol in utero have significant facial or other physical features, making FASD an invisible disability for the majority of individuals (Riley, Clarren, Weinberg, & Jonsson, 2011). In addition, obtaining an accurate maternal prenatal drinking history has made diagnosis a challenge, particularly for those with no physical characteristics, resulting in many individuals remaining undiagnosed. Inclusion of FASD in the most recent *Diagnostic and Statistical Manual*, 5th edition (DSM-5) as Neurobehavioral Disorder associated with Prenatal Alcohol Exposure (ND-PAE) may ultimately begin to address these issues, although FASD is currently listed only under Conditions for Further Study (Kable et al., 2016).

In this chapter, we begin by providing a description of FASD and its overlaps with other neurodevelopmental disorders, before moving on to a discussion of biomarkers, why they are needed, and a consideration of neuroethical issues surrounding the use of biomarkers in neurodevelopmental disorders in general and FASD in particular. As preface to our discussion of FASD, we present the voices of some adults living with FASD (Box 1). These words speak to the individuals' experiences, their insights, issues of stigma and shame, the value of having a diagnosis, and who they are as people. These words highlight the point that despite the range of adverse effects of prenatal alcohol exposure, the majority of adults with FASD can live successful and productive lives if they have a diagnosis and get the support and services they need.

2. Fetal alcohol spectrum disorder (FASD)

2.1 Phenotypic manifestations and diagnosis

Prenatal alcohol exposure can result in broad spectrum of adverse developmental consequences falling under the umbrella of FASD. Despite the recognition of fetal alcohol syndrome (FAS) over four decades ago, prenatal alcohol exposure remains a leading cause of developmental disabilities in North America and worldwide. FAS, which can occur with chronic exposure to high doses of alcohol, represents the most severe phenotypic manifestation of FASD (Jones & Smith, 1973; Jones, Smith, Ulleland, & Streissguth, 1973). The diagnostic criteria for FAS include pre- and postnatal growth restriction, a characteristic set of facial dysmorphologies, and central nervous system alterations, including neurological abnormalities,

BOX 1 Stories of adults with FASD.

From CJ Lutke, "Words of experience to teens and adults with FASD," NOFASD Australia (https:/www.nofasd.org.au)

"So you know a little about me, my name is CJ and I am 35 years old. I was diagnosed with fetal alcohol syndrome when I was a baby… my words are for all you teens and adults with FASD who have felt like somehow you don't belong, don't fit in, are not good enough, and are ashamed of having FASD because that is how you have been made to feel. Only, I am going to talk about how much we are worth. FASD does NOT define you—it is only ONE piece of who you are… You are NOT a FASD person—you are a person who has FASD. Person first. FASD second. And, I should add, everyone I know with FASD—and I know lots—no matter what 'bad' things they have done, is a good person who tries their very best, every day to fit in and do the best they can. The problems we all have are NOT just about us. They are about the rest of the world that doesn't understand us, doesn't believe us, and so doesn't help us the way we need help… I have had to learn that, because there are things I cannot do, it does NOT mean I am dumb or stupid or lazy or making excuses. It just means I am like every other person in the world. Some things I can do, some things I can only do with help, and some things I cannot do. And if you cannot do something—well, that is okay too. I cannot work full time, the best I can do is part time and only some of the time—or my life falls totally apart. So I do the best I can do and if others don't know it is my best, well, I have finally learned that is their problem, not mine."

Taylor's Story (CDC: FASDs: My Story)

Taylor was not diagnosed until 8th grade. He wrote about his experiences at age 23: "We were like so many other families out there. We were looking for guidance and trying to find counselors, practitioners. Through NOFAS, I was able to have a voice and speak out. By talking with others who are just at the beginning of their FASD journey, we are also healing and helping ourselves—by reminding us that we are not alone… Now, NOFAS has empowered me. Several times a year they give me the podium to speak and tell my story. Getting up in front of a crowd to speak about FASD takes away the control that this disability had over me. I didn't know why I did a lot of things I used to do. But with this, I do know why I am doing it. For the past year, I have been leading a teen group with the help of Kathy [Mitchell, Vice President, FASD United (formerly NOFAS)] and my dad. We mostly talk about what it's like to be us, our day-to-day life, things we run into. We get into how bad it feels to be misunderstood, how we just wish people would understand us. But at the end of the day, no matter how bad, we are all smiles because we've got together and met people just like us."

Frances' Story (CDC: FASDs: My Story)

"I do a lot of writing to express my feelings. It helps me. I also watch people very carefully to learn how to do certain things. I tend to read everything twice to comprehend what I am reading. For my anxiety, I avoid loud and crowded places. I always surround myself with people that I feel comfortable and safe

BOX 1 Stories of adults with FASD.—cont'd

with… I want people to know that there is hope. I keep telling myself, if I can survive, others can too. FASD comes with a lot of shame and challenges. I always tell people to stop and think before taking that drink. Pregnant women should remember that they are not drinking alone."

For more voices, see also: youtube.com: Individuals with an FASD, FASD United (formerly NOFAS); Centers for Disease Control and Prevention (CDC): FASDs: My Story (https://www.cdc.gov/ncbddd/fasd/stories).

Text taken from postings on the CDC and NOFASD Australia websites with permission.

developmental delays, and intellectual impairment (Stratton, Howe, & Battaglia, 1996). Exposure to alcohol at levels that do not produce full FAS can result in either partial FAS (pFAS), where only some of the diagnostic features occur, or in numerous alcohol-related effects that can be primarily physical (alcohol-related birth defects, ARBD) or primarily neurobehavioral (alcohol-related neurodevelopmental disorder, ARND), although ARBD and ARND are not mutually exclusive (Stratton et al., 1996). Neurobehavioral and neurodevelopmental deficits are consistently observed across the spectrum of FASD. As defined in the DSM-5 as ND-PAE, these deficits include neurocognitive impairment (cognitive function, learning and memory, executive function), impairment in self-regulation (attention, impulsivity, behavioral regulation, stress responsiveness, mood/affect, sleep abnormalities), and deficits in adaptive function (communication, social behavior, activities of daily living) (Astley et al., 2009; Carter et al., 2016; Doyle & Mattson, 2015; Lynch, Kable, & Coles, 2015; Panczakiewicz et al., 2016; Streissguth & O'Malley, 2000). Moreover, recent data suggest adverse outcomes in domains including metabolic changes, physiological dysfunction (e.g., endocrine, immune, etc.), and vulnerability to physical and mental diseases and disorders (Bodnar et al., 2020, 2018; Burd et al., 2007; Popova et al., 2017). Importantly, the adverse neurodevelopmental outcomes of children with FASD persist into adulthood, but if a diagnosis was not made in childhood, these outcomes may go unrecognized or undiagnosed (Coles et al., 2023; Famy, Streissguth, & Unis, 1998; Lemoine, Harousseau, Borteyru, & Menuet, 2003; Moore & Riley, 2015; Spohr & Steinhausen, 2008; Streissguth et al., 2004; Weyrauch, Schwartz, Hart, Klug, & Burd, 2017).

Given the broad umbrella of FASD, diagnoses are typically a multistep and complex process involving a multidisciplinary clinical team that can include developmental or behavioral pediatricians, clinical geneticists/dysmorphologists, and psychologists or neuropsychologists. As prenatal alcohol exposure is a required criterion in the absence of facial dysmophologies, diagnostic teams also require a skilled interviewer to assess maternal alcohol use. Unfortunately, however, even with the most skilled interviewer, an accurate maternal history is often unattainable. Finally, others involved in the assessment and support of individuals with FASD can include psychiatrists, speech pathologists, occupational therapists, physical therapists, special educators, audiologists, and/or ophthalmologists (Coles et al., 2023).

2.2 Genetic and environmental risk factors

The degree to which alcohol affects development depends on a variety of factors such as timing, pattern, and level of alcohol exposure, overall maternal health and nutrition, and genetic background, which may influence the disparity between maternal drinking rates and the prevalence of FASD (Pollard, 2007). Genetic risk may also play a role in susceptibility to FASD, as evidenced by studies in twins that have shown 100% diagnostic concordance in monozygotic twins (i.e., identical genetic code), but only 64% concordance in dizygotic twins (i.e., only 50% shared genetic code). However, the exact genes underlying susceptibility or resilience are unclear, as studies in human populations remain underpowered and confounded by risk genes for alcohol consumption (e.g., alcohol dehydrogenase genes). That said, this remains an active area of investigation using *in vivo* model systems, with identification of genes that could potentially modulate alcohol's effects on facial dysmorphologies, growth, and neurodevelopmental deficits (Kaminen-Ahola, 2020; Lussier, Petrelli, Hicks, & Weinberg, 2023; Sambo & Goldman, 2023). Importantly, environmental risk and resilience factors are equally critical to the impact of alcohol on development. These include prenatal factors such as maternal health, socioeconomic status, substance use, trauma, abuse, etc., and postnatal factors such as early diagnosis, adversities such as abuse or neglect, stability of the environment, nutrition, health care, environmental toxins or contaminants, and educational and social support (Flannigan et al., 2021). All of these factors can act in concert with prenatal alcohol exposure to influence physical, behavioral, and neurodevelopmental outcomes.

2.3 Overlaps between FASD and other neurodevelopmental disorders

The profile of individuals with FASD often includes other functional or phenomenologically described neurodevelopmental disorders, with the primary etiology being linked to a diagnosis of FASD and prenatal alcohol exposure. Major neurodevelopmental disorders that have phenotypic overlaps with FASD include intellectual disability (ID), autism spectrum disorder (ASD), and attention deficit hyperactivity disorder (ADHD). Due to overlaps among these disorders and the potential underreporting of prenatal alcohol exposure, FASD may sometimes be diagnosed as another neurodevelopmental disorder such as ADHD or ID alone, leading to poorer access to FASD-specific umbrella services.

2.3.1 Intellectual disability (ID)

ID is estimated to impact approximately 1% of children and manifests through a range of problems in mental abilities (Maulik, Mascarenhas, Mathers, Dua, & Saxena, 2011). Generally, ID presents through deficits in two main categories of mental ability: (1) intellectual functioning (learning, problem solving, judgment, etc.) and (2) adaptive behavior (social, conceptual, and practical skills such as communication, independence, etc.) (Patel, Apple, Kanungo, & Akkal, 2018; Patel, Cabral, Ho, & Merrick, 2020). Of particular relevance to the present chapter, the most common environmental source of ID is prenatal alcohol exposure, with ID frequently manifesting in children with FASD (Greenspan, Brown, & Edwards, 2015; Greenspan & Novick Brown, 2022). For a more thorough discussion of the ethical implications of ID versus FASD diagnoses from a legal and clinical perspective, please refer to Greenspan and Novick Brown (2022).

2.3.2 Autism spectrum disorder (ASD)

ASD is estimated to impact approximately 2% of children (1 in 50), and typically manifests through a spectrum of phenotypes relating to deficits in social communication, as well as challenges with restricted interests and repetitive behaviors (Hyman et al., 2020). ASD and FASD share deficits in social and communicative functioning (Stevens, Nash, Koren, & Rovet, 2013), socially inappropriate behaviors and difficulty with peers (Bishop, Gahagan, & Lord, 2007), as well as hyperactivity, impulsivity, emotional lability, and difficulty changing strategies or inflexibility (Harris, MacKay, & Osborn, 1995). Several lines of evidence also suggest that FASD and ASD

may be co-occurring (Harris et al., 1995; Lange, Rehm, Anagnostou, & Popova, 2018; Nanson, 1992; Popova, Lange, Shield, et al., 2017), indicating that behavioral alterations characteristic of ASD may also be present in children diagnosed with FASD.

2.3.3 Attention deficit hyperactivity disorder (ADHD)

ADHD is one of the most common neurodevelopmental disorders, affecting an estimated 8.4% of children and 2.5% of adults (Danielson et al., 2018; Simon, Czobor, Bálint, Mészáros, & Bitter, 2009). Characterized by symptoms of inattention, hyperactivity, and impulsivity, ADHD can have profound deleterious impacts on interpersonal relationships and daily functioning, and can lead to poorer self-worth and mental health (Austerman, 2015). Several disorders show similar symptoms or co-occur with ADHD, including learning disorders, mood disorders, and ASD (Hours, Recasens, & Baleyte, 2022). Perhaps most importantly, children and adolescents with FASD commonly have deficits in attention that are reminiscent of those observed in ADHD, which can lead to missed or incorrect diagnoses when information about prenatal alcohol exposure is not available (Burd, 2016; Rasmussen et al., 2010).

2.3.4 Implications

Beyond potential issues of resource access, children with FASD may not adequately respond to treatments or drugs used to treat other neurodevelopmental disorders. For instance, while children and adolescents with FASD commonly have deficits in attention that may appear similar to those observed in ADHD (Greenspan & Novick Brown, 2022), the drugs commonly used to treat ADHD may not perform as expected in individuals with FASD. Indeed, children with FASD often respond differently from those without FASD to methylphenidate (e.g., Ritalin) and dexamphetamine (e.g., Adderall), where those drugs do not show the expected paradoxical effects (Burd, 2016; Peadon & Elliott, 2010). Rather, evidence from animal models suggests that prenatal alcohol exposure increases sensitivity to amphetamines, which are the basis for ADHD medications such as Adderall (Uban et al., 2015). These findings point to the critical importance of distinguishing between FASD and other potential neurodevelopmental disorders to provide appropriate care and treatment to children and adolescents, thus ensuring that they develop appropriately and thrive in their everyday lives.

3. Biomarkers of FASD

It is difficult to estimate the true prevalence of the full spectrum of FASD in the population (Roozen et al., 2016). However, recent studies suggest that FASD is more prevalent than previously recognized. CDC studies from 2002 suggested that FAS occurs in approximately 1 infant per 1000 live births in certain parts of the United States (CDC, 2002). Further in-person assessments of school-aged children in several communities (May et al., 2014, 2009) reported higher estimates (up to 6–9 per 1000 children) of FAS. More recently, using active case-ascertainment methods, data suggest that the full spectrum of FASD may be as high as 1–5% of the population (May et al., 2018), which is almost twice the prevalence of autism spectrum disorder (~2%) and many times the prevalence of other neurodevelopmental disorders, including cerebral palsy and Down Syndrome. Studies using similar methods in the United States, Canada, Italy, Poland, and Croatia similarly reported an FASD prevalence as high as 5% in the general population (May et al., 2014, 2011, 2009, 2004, 2021, 2015; Okulicz-Kozaryn, Borkowska, & Brzózka, 2017; Petković & Barišić, 2013; Popova et al., 2019; Popova, Lange, Probst, Gmel, & Rehm, 2018). While this estimated prevalence may not be generalizable to all communities, the data certainly suggest a greater prevalence of FASD than previously recognized. Moreover, an estimated half of women under 30 years of age in the United States have unplanned pregnancies (May et al., 2004), and thus may continue to consume alcohol during the first trimester while pregnancy is unrecognized. As well, it is estimated that 10–15% of women in Canada and the United States continue to drink throughout pregnancy, with approximately 3% continuing to binge drink, which is particularly deleterious to fetal development (Bonthius & West, 1990; Popova, Lange, Probst, Gmel, & Rehm, 2017).

For children with FAS, their characteristic facial dysmorphology along with other physical, cognitive, and behavioral/adaptive issues, make diagnosis possible, particularly if seen by trained clinicians. By contrast, a large majority of children on the FASD spectrum may show no physical evidence of alcohol exposure, and without a confirmed maternal history for prenatal alcohol exposure, this etiologic diagnosis may be missed (Chasnoff, Wells, & King, 2015). Indeed, in the study noted above (May et al., 2018), only 2 of 222 children had been diagnosed previously. This gap reinforces the urgent need to develop methods for identifying individuals exposed to alcohol in utero, as early intervention is known to reduce adverse consequences.

3.1 What are biomarkers?

This brings us to the issue of biomarkers (i.e., biological markers). The Biomarkers Definitions Working Group (2001), convened by the NIH Director's Initiative on Biomarkers and Surrogate Endpoints, states that a biomarker is "a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" (p. 91). In their monograph on biomarkers in environment risk assessment, the WHO (World Health Organization and International Programme on Chemical Safety, 1993) states that the term biomarker is "used in a broad sense to include almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction. In the assessment of risk, biomarkers may be used in hazard identification, exposure assessment and to associate a response with the probability of a disease outcome" (p. 11). Another definition was developed by the National Institute of Environmental Health Sciences (2023), which defines a biomarker as "an objective measure that captures what is happening in a cell or an organism at a given moment." They further state that "biomarkers play an important role in illuminating relationships among environmental exposures, human biology, and disease" (p. 1). Importantly, biomarkers must be highly sensitive and specific, meaning they must correctly detect individuals who have the disorder (sensitivity) and reject healthy individuals without the disorder (specificity) (Ray, Manach, Riou, Houle, & Warner, 2010).

The need for sensitive, specific, and reliable biomarkers of prenatal alcohol exposure has long been recognized in the FASD field (Allen, Sillanaukee, Strid, & Litten, 2003; Bager, Christensen, Husby, & Bjerregaard, 2017; Bakhireva & Savage, 2011; Bearer, Stoler, Cook, & Carpenter, 2004). Biomarkers could allow for early identification of an infant or child who has been exposed to alcohol but lacks characteristic facial or other physical features that would facilitate diagnosis, thus allowing for early interventions that may attenuate alcohol's adverse effects (Bearer et al., 2004). Biomarkers could also aid in identifying children who present with cognitive, behavioral, or adaptive problems as they grow but who had not been identified previously, providing support for more targeted interventions. Finally, biomarkers could be useful in allowing for education

and support to help women reduce or stop using alcohol during pregnancy, as well as to mitigate adverse effects in subsequent pregnancies (Bakhireva & Savage, 2011; Bearer et al., 2004).

Based on these goals, biomarkers of FASD generally fall into two categories: (1) those that detect the presence of alcohol or prenatal alcohol exposure, and (2) those can predict or are associated with child neurodevelopmental outcomes. To date, the majority of research and clinical applications have focused on the first category, though the clinical implementation of most biomarkers remains challenging and extremely limited. While in early stages, research aimed at developing novel biomarkers to predict neurodevelopmental outcomes have begun to highlight some promising approaches.

3.2 Biomarkers of alcohol exposure and metabolism

Maternal self-report is the oldest and most widely used method of assessing prenatal alcohol exposure, and several instruments have been shown to be sensitive in assessing a history of alcohol intake (Russell et al., 1996), including the TWEAK (Russell, 1994), the Alcohol Use Disorders Identification Test (AUDIT), the Michigan Alcohol Screening Test, and the CAGE (Ewing, 1984). However, factors such as lack of recall for amount and frequency of intake, and/or reluctance to report truthfully due to the stigma surrounding alcohol and pregnancy can reduce their usefulness. The use of biomarkers measured from biological samples, either alone or in combination with maternal self-report, would provide a more objective approach. A number of biomarkers involving direct or indirect products of ethanol metabolism measured in biological specimens from both the mother and infant (e.g., urine, blood, meconium) have been utilized in research and clinical settings.

While ethanol and its major metabolite acetaldehyde would be the most direct indicators of alcohol exposure, their short half-lives reduce their usefulness. However, other biomarkers of alcohol exposure have been developed; these can be measured from blood to provide an indication of alcohol consumption levels in adults, and include γ-glutamyl transferase (GGT), mean erythrocyte macrocytic volume (also mean corpuscular volume) (MCV), and carbohydrate deficient transferrin (CDT) (Bager et al., 2017; Bakhireva & Savage, 2011). While found throughout the body, GGT is primarily a liver enzyme that has been used to assess liver dysfunction

or bile duct conditions that can be caused by alcohol (Allen et al., 2003). This marker is now also commonly used to assess risk of coronary heart disease, type 2 diabetes and stroke (Koenig & Seneff, 2015). Elevated MCV can result from adverse effects of alcohol on erythroblast development. Alone, MCV is considered a low sensitivity measure, but may be useful when assessed together with other markers (Allen et al., 2003; Bakhireva & Savage, 2011). CDT is a modified form of the iron transport protein transferrin, and is considered one of the most specific biomarkers of heavy alcohol consumption (Fagan et al., 2014). CDT levels remain elevated for ~2–3 weeks following moderate alcohol consumption and normalize within 2–4 weeks of abstinence (Allen et al., 2003). However, CDT has relatively low sensitivity, particularly in women or individuals with an elevated BMI (Allen et al., 2003; Fagan et al., 2014), and thus, should be used cautiously. With these considerations, it has been suggested that panels of two or more of these biomarkers might be needed to improve their utility (Allen et al., 2003; Bearer et al., 2004).

Substantial progress has also been made in identifying markers that focus on products of alcohol metabolism. For example, fatty acid ethyl esters (FAEEs) are a large group nonoxidative alcohol metabolites (esterification products of fatty acids and ethanol) (Bakhireva & Savage, 2011). They begin to form early in pregnancy and accumulate in the fetal gut from ~ the 20th week of gestation until birth, thus reflecting alcohol exposure in the second half of pregnancy (Bager et al., 2017; Bakhireva & Savage, 2011). Meconium FAEEs appear to identify both moderate and binge drinking patterns and may serve as a biomarker of developmental delays in children (Bakhireva et al., 2014; Bakhireva & Savage, 2011). The concentration of FAEEs has been shown to correlate with blood alcohol concentrations, and FAEEs may, in part, mediate ethanol-induced organ damage (Doyle et al., 1994). However, the specificity of meconium FAEE is inconsistent between different cohorts and FAEEs, potentially due to the small number of cases in studies to date (Bakhireva et al., 2014; Bearer et al., 2003, 1999, 2005; Kwak, Han, Choi, Ahn, Kwak, et al., 2014; Kwak, Han, Choi, Ahn, Ryu, et al., 2014; Ostrea et al., 2006). By contrast, FAEEs are also deposited in hair where they are stable, providing a long diagnostic time window for analysis and relatively high accuracy of prior alcohol exposure (Pragst & Yegles, 2008).

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are alcohol metabolites produced in the liver that can be measured in peripheral samples. EtG appears to be a sensitive biomarker of alcohol consumption, detectable in

blood for 4–8 h and in urine for up to 5 days after a drinking episode (Bager et al., 2017; Bakhireva & Savage, 2011). Sensitive methods for detecting EtG in hair have also been developed, providing another approach for assessing alcohol exposure. EtS is similarly detectable in serum for 4–8 h, and may be detectable in urine for up to 30 h after alcohol consumption (Bager et al., 2017). Finally, phosphatidyl-ethanol (PEth), a phospholipid formed in the presence of alcohol, is detectable in blood up to 6 weeks after sustained alcohol consumption (Allen et al., 2003). However, the validity of PEth as a biomarker has not been well established in pregnant women (Bakhireva & Savage, 2011). Importantly, for assessment of *in utero* exposure to alcohol, because these markers are found in a restricted timeframe shortly after birth, their use in later-life diagnoses is limited (Cabarcos, Álvarez, Tabernero, & Bermejo, 2015).

3.3 Biomarkers of neurodevelopmental outcome

The issues raised above point to the need for sensitive biomarkers that are not linked temporally to alcohol intake. To be useful, such biomarkers should be measurable in women and associated with child outcomes, and/or measurable in children and associated with neurodevelopmental, neurobehavioral, and other critical outcomes, particularly in the absence of facial dysmorphology or other physical characteristics of prenatal alcohol exposure. To this end, studies have begun to investigate neurobehavioral, neurobiological, physiological, imaging, genetic/epigenetic and other molecular measures to identify profiles or signatures specific to FASD. Here, we briefly review some of these new emerging research areas. However, we must keep in mind that numerous factors can affect the outcome in any domain, including dose, timing and pattern of alcohol exposure, genetics, and a myriad of other prenatal or postnatal environmental factors that all influence or modulate the effects of prenatal alcohol exposure.

3.3.1 Neuroimmune profile

Substantial evidence indicates that prenatal alcohol exposure results in alterations in immune/neuroimmune responsiveness and function by reprogramming immune activity in both the central nervous system (CNS) and periphery (Noor & Milligan, 2018). Neuroimmune activation in the developing brain results in activation of microglia, production of proinflammatory molecules, and alteration in gene expression (Drew & Kane, 2014). Such changes can alter normal neurogenesis, differentiation, migration, and survival of neurons and glia, thus disrupting normal brain

development, with long-term consequences, including changes in cognition, behavior, and health (Noor & Milligan, 2018). However, research to identify unique or specific immune signatures of prenatal alcohol exposure is relatively recent. Much of this work has utilized animal (primarily rodent) models, with important findings that set the stage for more recent exploration of immune/neuroimmune biomarkers of FASD in clinical populations.

Studies utilizing animal models have revealed a specific immune signature in offspring prenatally exposed to alcohol. For example, female rats exposed to moderate alcohol levels in utero exhibited unique cytokine profiles compared to controls during infancy, when microglia are more actively producing cytokines (Bodnar, Hill, & Weinberg, 2016). This suggests the possibility that microglia might be more activated following alcohol exposure, with long-lasting impacts on brain structure and function. These findings have significant clinical implications, as a considerable proportion of individuals diagnosed with FASD do not display physical indicators of alcohol exposure yet in many cases may remain as vulnerable to impaired CNS function as those diagnosed with FAS (Noor & Milligan, 2018). Indeed, the translational potential of this animal model research was realized in subsequent studies with cohorts of women and children. For example, evaluation of networks of interacting cytokines in people during pregnancy found distinct networks of activated or inhibited cytokines that were associated with both maternal alcohol consumption and child neurodevelopmental outcome (neurodevelopmental delay versus typical neurodevelopment) (Bodnar et al., 2018). Similarly, in a cohort of young children (Bodnar et al., 2020), analyses revealed differential cytokine network activity associated with both prenatal alcohol exposure and neurodevelopmental status (delay versus typical development). In other words, unique immune profiles in alcohol-exposed compared to unexposed children were noted, irrespective of neurodevelopmental status, reflecting alcohol exposure *per se*. As well, alcohol-exposed children who were typically developing had differentially activated cytokine networks compared to alcohol-exposed children who had developmental delay.

The ability to identify alterations in maternal and/or child cytokine networks that can be associated with both alcohol exposure and neurodevelopmental delay provides a critical first step toward the development of possible biomarkers for early identification of at-risk children based on both maternal and child immune profiles. This is particularly important for those children who show no measurable physical phenotypes but whose

neurodevelopment has been adversely impacted by alcohol. Such biomarkers could then aid in developing more targeted early interventions for children who are at risk. Furthermore, this research highlights the potential for novel therapeutic approaches using immunomodulatory agents in intervention and treatment for FASD. Indeed, animal model studies have shown that the adverse effects of alcohol on CNS glial activation, which in turn lead to increased cytokine production, can be attenuated by a peroxisome proliferator-activated receptor gamma (PPARγ) agonist that acts as an antiinflammatory agent by blocking microglial activation (Drew & Kane, 2016). These findings may also point to the potential use of these biomarkers as measures of treatment response, whereby children who respond to treatment may show immune signatures that become more similar to those found in typically developing children.

3.3.2 MicroRNA (miRNA) signatures

miRNAs comprise a family of small molecules that regulate gene expression and cellular functions. Importantly, several studies suggest that miRNAs are sensitive to alcohol, mediate many of the effects of alcohol, and are involved in the regulation of genes involved in development (Balaraman et al., 2016; Salem et al., 2020; Tseng et al., 2019; Wang et al., 2009). Moreover, miRNAs are secreted into the blood and thus may circulate in the body (Hunter et al., 2008). During pregnancy, fetal tissues like the placenta also secrete miRNAs that may enter the maternal circulation (Mahnke et al., 2021). Thus, maternal plasma miRNAs have a dual origin and may serve as composite biomarkers for both maternal and fetal health (Balaraman et al., 2016).

To this end, a recent study investigated whether changes in plasma miRNAs in women consuming alcohol during pregnancy could predict infant outcomes (Balaraman et al., 2016). These analyses identified 11 miRNAs that were significantly elevated in the maternal plasma of those who consumed alcohol and whose children showed adverse effects relative to both women who abstained and those who consumed alcohol but whose children were apparently unaffected. When these miRNAs were combined with smoking history and socioeconomic status into a single predictor, the accuracy of classification into these three distinct groups was above 80%. These data suggest that maternal plasma miRNAs may be useful in predicting infant outcomes, and in case finding of individuals at risk for FASD. In a subsequent study of circulating miRNAs in infants (Salem et al., 2020), prenatal alcohol exposure was associated with alterations in

patterns of miRNA expression at both 2 weeks and 6.5 months of age compared to those in control infants. Importantly, these altered miRNA networks were shown to identify infants who at 6.5 months exhibited alcohol-related growth deficits and deficits in infant memory, independent of measures obtained from the mother. These findings support the potential of miRNAs as biomarkers for predicting developmental problems linked to prenatal alcohol exposure.

3.3.3 Epigenetic signatures

Although FASD is a consequence of prenatal exposure to alcohol, both genetic and environmental factors contribute to the complex FASD phenotypes of growth deficits, physical abnormalities, physiological alterations, and neurocognitive, neurodevelopmental, and neurobehavioral impairments (Kaminen-Ahola, 2020). Increasing evidence suggests that epigenetic mechanisms may be one of those factors involved in mediating the broad range of effects that can occur following prenatal exposure to alcohol, and numerous studies have now begun to investigate epigenetic mechanisms in the etiology FASD. Importantly, epigenetic marks are emerging as potential biomarkers or signatures of early-life exposures, such as prenatal alcohol exposure. Generally, epigenetics refers to modifications of DNA and its regulatory components that potentially modulate gene transcription and changes in gene expression, without changing underlying DNA sequences (Aristizabal et al., 2020). The most widely studied epigenetic modification in human populations is DNA methylation, as it is both stable over time and responsive to environmental and genetic influences. Thus, DNA methylation is the focus of the studies discussed here.

Several *in vitro* or animal model studies identified epigenetic changes induced by prenatal or early postnatal exposure to alcohol. Ethanol-related changes were found to occur in specific brain regions or throughout the entire brain, with evidence of both decreased and increased DNA methylation, depending on the paradigm, brain area studied, and biological sex (Laufer et al., 2013; Liu, Balaraman, Wang, Nephew, & Zhou, 2009; Lussier, Bodnar, et al., 2018; Lussier et al., 2021; Lussier, Weinberg, & Kobor, 2017; Marjonen et al., 2015; Ungerer, Knezovich, & Ramsay, 2013). These studies paved the way for translational work with human cohorts. An early study characterized the DNA methylation profile in buccal epithelial cells (BECs) from a small cohort of children with FASD ($n = 6$), and found unique alterations in DNA methylation profiles, particularly

within the protocadherin gene clusters, that were influenced by sex and medication exposure (Laufer et al., 2015). DNA methylation profiles were subsequently studied in BECs from a large cohort ($N = 206$; 110 FASD and 96 controls) of children recruited by NeuroDevNet (now the Kids Brain Health Network), Canadian Networks of Centres of Excellence, where a DNA methylation signature of 658 loci specific to children with FASD was identified (Portales-Casamar et al., 2016). Although these data were collected from BECs, the vast majority of differentially methylated genes was highly expressed in the brain, and these alterations occurred in several genes previously implicated with prenatal alcohol exposure and altered neurodevelopment. To validate these findings, genome-wide DNA methylation profiles of BECs from an independent cohort of 24 individuals with FASD and 24 matched controls were identified, where 161 of the 658 loci from the original NDN cohort were replicated (Lussier, Morin, et al., 2018).

Importantly, the DNA methylation data from the NDN cohort were successfully utilized to generate a predictive algorithm to classify individuals as FASD or control with high accuracy (83% accuracy in predicting FASD compared to control status in the test cohort of 48 individuals [91% accuracy in the training dataset]) ((Lussier, Morin, et al., 2018)). Importantly, this model also showed 99% specificity in predicting ASD cases as non-FASD in a second independent dataset, highlighting the potential for DNA methylation as a sensitive and specific predictor of other neurodevelopmental disorders.

Building on this work, a recent epigenome-wide association study on FASD aimed to further to uncover loci associated with specific features of the FASD phenotype (Cobben et al., 2019). Utilizing a discovery set of 39 individuals with FASD and 64 controls, and a replication set of 7 well-characterized FASD cases and 28 controls, the investigators found suggestive evidence for differentially methylated regions associated with the FASD phenotypic features of impaired growth, facial features and CNS development. These findings suggest that DNA methylation data could be used to identify specific sets of phenotypes linked to FASD, beyond the simple identification of exposed versus unexposed individuals. Together, these studies demonstrate that unique epigenetic profiles can be identified in individuals with FASD, and that such profiles could form a basis for the development of epigenetic biomarkers that could facilitate early diagnosis, intervention, and support for individuals with FASD.

3.3.4 Other potential tools

Although we outline several avenues for biomarkers of prenatal alcohol exposure and FASD above, this remains an active area of investigation with numerous other types of markers and predictors in development. A few recent examples are briefly described:

Eye tracking measures have been used in a small cohort of children to distinguish children with FASD, ADHD, or typical development with relatively good accuracy using several features obtained from a short testing session (Tseng et al., 2013). Importantly, eye tracking differences can accurately differentiate children with FASD versus ADHD, which represents an important step forward in distinguishing these two neurodevelopmental disorders.

The *cardiac orienting response (COR)* has been noted as a potential biomarker to assess the effects of prenatal alcohol exposure on infants (Mesa et al., 2017). The COR is characterized by a specific pattern of heart rate deceleration in the presence of novel stimuli and results from the heart gating oxygen to the central nervous system. Of note, animal model studies suggest that the COR may provide an early index of the efficiency of prefrontal cortical functioning. Thus, a recent study compared the ability of the positive and negative predictive values of the COR and the Bayley Scales of Infant Development-II to identify developmental delay in children tested in a habituation/dishabituation learning paradigm. The COR performed slightly better than the Bayley at classifying children as alcohol-exposed or unexposed, suggesting that the possibility that the COR could be a useful screening tool for identifying different neurobehavioral profiles of FASD.

Imaging studies represent yet another emerging area of possible biomarker development. Two recent studies utilizing functional near-infrared spectroscopy (fNIRS) measured blood oxygenation in the prefrontal cortex during an executive function task requiring cognitive inhibition (Barrett, Kable, Madsen, Hsu, & Coles, 2019) and a task that elicits frustration (Kable, Coles, & CIFASD, 2017) in children with FASD compared to unexposed controls and children with other neurodevelopmental or behavioral problems. Together, the results from these two studies suggest that oxygen utilization is altered in children with FASD during both cognitive and emotional tasks related to PFC function, and that these alterations differentiate children with prenatal alcohol exposure from those with other clinical conditions. Recent MRI studies also show promise in the development of biomarkers of FASD. For example, a recent study (Andre et al., 2020) examined the effects of prenatal alcohol exposure on both brain

structure and mental health outcomes in children. Importantly, as children prenatally exposed to alcohol are more likely than unexposed children to experience early-life adversity, the effects of prenatal alcohol were assessed in the presence or absence of adverse postnatal exposures. The results demonstrated that, overall, prenatal and postnatal exposures may differentially interact with alcohol to influence brain development.

Together, the studies discussed above reflect the rapid advancement of methods to predict and screen for FASD, and to distinguish FASD from other exposures and neurodevelopmental disorders, further emphasizing the need for discussions around ethical considerations for the development and implementation of biomarkers.

4. Stigmatization in FASD

Biomarkers have the potential to increase the identification of people with, or at risk for, FASD, as well as drawing attention to the behavior of biological mothers. This next section will review key concepts concerning stigma and how increased use of biomarkers in clinical settings might impact individuals with FASD and their families, including the potential to cause or increase stigma for these individuals. We will then note areas and approaches that could be leveraged to mitigate stigma in the future.

Stigma is a key consideration when discussing the development and implementation of biomarkers, as missed diagnoses of FASD can have profound societal and personal implications that go beyond medical impacts. Indeed, consequences of stigma overall can considerably increase stress and anxiety, as well as feelings of isolation and despair.

Stigmatization has been defined as a "social and culturally constituted process whereby a person is first identified as different and then devalued, leading to status loss and discrimination" (Roozen, Stutterheim, Bos, Kok, & Curfs, 2020) (p. 754). A similar definition by Goffman (1963) describes stigma as "an attribute that makes a person different from others in a social category, and it reduces the person to a […] discounted status […]; it is made up of a discrepancy between the virtual (or perceived) identity and the actual identity of the stigma's bearer" (p. 133). Four primary types of stigma have been characterized and will be discussed here (Hall, Hall, & Cockerell, 2011; Roozen et al., 2020): (1) public stigma, which represents the responses or reactions to a person perceived to have a stigmatized condition (Bos, Pryor, Reeder, & Stutterheim, 2013); (2) self-stigma, which represents internalization of the negative beliefs and attitudes about an

individual, reflecting the social and psychological impact of having a stigma; (3) stigma by association, which extends to individuals connected or associated with those who have the stigmatized condition, and can include the social and psychological reactions to being associated with a stigmatized person; and (4) structural stigma, which results from and is perpetuated by society's institutions and systems (Bos et al., 2013). It results from structures within society that marginalize people and increase negative attitudes and judgment. While these are interrelated and share common threads, public stigma is considered to be at the core of the other three types.

Individuals with disabilities have experienced extensive stigmatization over the centuries. One recent turning point in the United States came with the passage of the Americans with Disabilities Act in July 1990, which resulted in considerable gains for disabled individuals. Unfortunately, however, there are still significant barriers or disadvantages in "…education, employment, income, housing, transportation, and other eco-social determinants of health," as well as "disparities in … health-care services" (Parekh & Childs, 2016) (p. 4) for individuals with disabilities, who are seen as different and therefore devalued, discounted, and often excluded.

4.1 FASD and stigma

For FASD, the diagnosis itself contributes to stigma as the term FASD identifies both the cause and the nature of the condition (Choate & Badry, 2019). If, like other disabilities that are often named for the person who identified or described them rather than for their cause, FASD had been named differently, the stigma associated with the diagnosis might have been reduced, at least to some extent. Compounding this issue is the contradictory fact that a large majority of individuals prenatally exposed to alcohol show no physical evidence (i.e., facial dysmorphology, birth defects, growth deficits) of alcohol exposure, making diagnosis more difficult, as well as the acceptance of an individual with FASD as someone with a disability. Indeed, FASD is often called the invisible disability (Riley et al., 2011). See Box 2 for an account of the stigmatization experienced by a person with FASD.

4.1.1 Public stigma

Public stigma related to FASD can occur at all levels and can have long-term and far-reaching consequences (Choate & Badry, 2019; Corrigan et al., 2017; Green, Cook, Racine, & Bell, 2016; Roozen et al., 2020). For one, individuals who consume alcohol during pregnancy may be stigmatized

BOX 2 Experiencing stigma in FASD.

From CJ Lutke, "Words of experience to teens and adults with FASD," NOFASD Australia (https:/www.nofasd.org.au)

As one of the Adult Leadership Committee for FASD Change Makers, I speak up because not speaking out when you have FASD means we think it is something shameful—and you and me—we—have NOTHING to be ashamed of. Nothing at all…. But stereotyping is not just about labels—it is also about how we function and other people's misunderstandings get in the way…. Our efforts should be valued based on our efforts—how hard we try every day—and WE—you and I—can be PROUD of those efforts. We have to work many times harder than anyone else to do what others take for granted. Be PROUD. We are NOT disposable. This is our world, too. We are STRONG; we just don't know it until we figure it out. We have much to give, much to share, and much to teach… Because for us, FASD is not just a disability, it is a life experience. And no one understands that experience better than us…. FASD does not make us "hopeless"; it does not make us "tragic"; and it does not make us "victims." Those are someone else's labels and I reject them; we should all reject them. FASD just makes us different and there is nothing wrong with that. We should not let their labels or their discomforts define us, make us feel ashamed or cause us to be silent. That just devalues us—and that is what causes the shame we feel because it makes us feel worthless and it makes us doubt our own true self.

Text taken from postings on the NOFASD Australia website with permission.

as bad or neglectful parents, as being secretive or in denial about their alcohol dependence, or as responsible for adverse outcomes in their children (Corrigan et al., 2017). They may be blamed or shamed for consuming alcohol during pregnancy. Consistent with this is the often-heard statement that FASD is completely preventable. For example, a publication by the American Academy of Pediatrics states as one of its key premises: "Alcohol-related birth defects and developmental disabilities are completely preventable when pregnant women abstain from alcohol use" (Williams, Smith, & Committee on Substance Abuse, 2015) (p. e1395). Although factually true in the sense that FASD results from prenatal exposure to alcohol, this premise completely ignores the underlying trauma, social inequalities, mental health challenges or other social and environmental factors that may underlie substance use, and/or the fact that women may not be aware that they are pregnant when they drink (Abadir & Ickowicz, 2016; Corrigan et al., 2017). Indeed, studies have found that the highest risk of

having children with FASD came from individuals with lower socioeconomic backgrounds and lower levels of education, inadequate nutrition during pregnancy, alcohol-using partners, reduced access to prenatal care, and increased stress and/or abuse during pregnancy (Abadir & Ickowicz, 2016; Choate & Badry, 2019; Salmon, 2008), highlighting the wide range of environmental factors that contribute to both maternal substance use and adverse outcomes in the children as targets for prevention and intervention.

Moreover, individuals with FASD themselves are often stigmatized because of their disabilities. Some may consider them a burden on society due to their medical and other problems (Roozen et al., 2020). Further, for the majority of individuals with no identifying physical features of prenatal alcohol exposure, their cognitive, behavioral, and adaptive issues may be viewed as purposefully bad or lazy behavior (Choate & Badry, 2019).

Biological parents may also feel ashamed and thus conceal or deny drinking or avoid seeking counseling or treatment for themselves and help for their children. Even nonbiological parents of a child with FASD may be stigmatized. They may be held responsible for their child's behavior and other issues that are part of the disability, and thus feel misunderstood as well as shamed and blamed as bad parents. This, in turn, can impede their ability to seek help from support networks or professionals for their children, leaving their children undiagnosed and without the support and help they need.

4.1.2 Self-stigma

Self-stigma in relation to FASD also comprises several issues that can adversely impact parent-child relationships and prevent both parents and their children from seeking and/or getting help and support (Roozen et al., 2020). Children with FASD often internalize negative beliefs, which may affect their confidence and self-esteem and cause them to view themselves as less able and capable than they really are. Biological parents of children with FASD may also feel shame and blame, regardless of the fact that no one drinks during pregnancy with the intent to harm their child, but rather, that alcohol use and addiction is a disorder with multiple biological and social/environmental underpinnings. Many people are also unaware of the adverse effects of prenatal exposure to alcohol or may have never been told that drinking during pregnancy would be harmful.

4.1.3 Stigma by association

Stigma by association can extend to individuals connected or associated with those with FASD, and possibly also to those connected with individuals who consumed alcohol during pregnancy (Roozen et al., 2020). These individuals experience negative social and psychological reactions similar to those experienced by a stigmatized person. For instance, biological parents may experience being stigmatized not only for having consumed alcohol but also because they are associated with a child with FASD. Stigma by association is also felt by nonbiological parents. The consequences of stigma by association significantly increase stress and anxiety, and feelings of isolation and desperation, and can adversely affect outcomes for both parents and children (Choate & Badry, 2019).

4.1.4 Structural stigma

Structural stigma is also associated with FASD. For example, procedures or processes initiated to educate people about the adverse effects of prenatal alcohol exposure or that aim to reduce drinking in general may include punitive measures, emphasize risk and responsibility, and overlook systemic social and environmental conditions that contribute to substance use (Roozen et al., 2020). Such structural issues may marginalize people and increase negative attitudes and judgment. Providing support and services for parents or caregivers and for individuals with FASD can begin to overcome issues resulting from structural stigma.

4.2 Reducing stigma in FASD

Reducing the stigma and shame surrounding FASD is essential, and it has been suggested that multilevel approaches targeting all levels—individual, interpersonal, community, organizational, and structural—are needed. Despite the fact that it has become a public health priority to educate people about the adverse effects of consuming alcohol during pregnancy, stigma and health literacy have impacted the effectiveness of public health campaigns (Corrigan et al., 2018).

One potential solution may be to increase health literacy around FASD. Health literacy refers to "… the degree to which individuals accurately understand the impact of a health condition and interventions meant to remediate it" (Corrigan et al., 2018) (p. 267). In other words, the more one knows and understands about a condition, the less likely one may be to stigmatize it and the more likely one may be to engage in prevention

strategies (Corrigan et al., 2018). To this end, a recent study found that those with higher FASD literacy scores were more likely to agree that FASD is a major public health concern, and that women should not consume alcohol during pregnancy, providing support for the need for more public education about FASD.

4.2.1 Public perception

One factor underlying the public misperception of FASD is that literature and public information about FASD focus more on prevalence and problems from a biomedical perspective, rather than on intervention and support that could help to facilitate positive outcomes (Choate & Badry, 2019). In other words, a strength-based rather than a deficit-based approach to viewing and describing FASD could result in a shift that "…increase(s) the efficacy of the individual worth, the value of intervention and the empowerment of people and families addressing the life course challenges of FASD" (Choate & Badry, 2019) (p. 44). Consistent with this approach, and in parallel with the disability movement more generally, there is a growing movement within the FASD community that advocates "nothing about us without us" (Choate & Badry, 2019) (p. 37). Specifically, the voices of individuals with FASD are challenging the negative perceptions and highlighting the positive outcomes that are possible (Choate & Badry, 2019). Indeed, this is a pillar of neuroethics research—bringing the voices of people with living experience to the forefront. An exemplar of this approach is the Vancouver International FASD Conference run by University of British Columbia Interprofessional Continuing Education for three decades, until they were halted in 2020. These conferences brought together laboratory and clinical researchers, professionals, and experts from a broad range of disciplines (e.g., social work, psychology, medicine, nursing, education, addiction, prevention, diagnosis), individuals from justice, government and policy, and individuals in the FASD community as the voices of living experience who were actively involved at every stage in the design, development, and implementation of the conference. The impact of the interactions, sharing, and learning that occurred at this conference have significantly changed attitudes and thinking and have moved the field forward, highlighting the power of multidirectional and inclusive interactions.

While further research is needed to determine which approach will have the greatest impact on reducing stigma around FASD, these findings support the need for education to disseminate accurate information on FASD.

4.2.2 Interpersonal contact

Beyond education, interpersonal contact is a second important strategy for reducing stigma. Empirical studies and meta-analyses of the literature have compared the effectiveness of both education and contact interventions in reducing stigma in the area of mental health (reviewed in Roozen et al., 2020). While one study found that both types of interventions, separately and together, had effects on stigmatizing attitudes, a number of others found that contact with a person with mental illness was more effective than educational interventions. Although there are no studies comparing education and contact intervention strategies in relation to FASD, medical students who had courses on substance use dependence in pregnancy or who worked in a clinic where they had direct contact with individuals with substance use dependence were less judgmental and more comfortable working with these individuals (reviewed in Roozen et al., 2020). These findings speak to the possibility that contact with individuals with FASD could similarly alter attitudes and reduce stigma for these individuals and their families.

4.2.3 FASD as a whole-body disorder

Finally, shifting the emphasis from the cognitive and behavioral outcomes in FASD to viewing FASD as a whole-body disorder is an emerging strategy to combat stigma (Choate & Badry, 2019). Increasing evidence demonstrates that beyond brain and behavior, FASD is a disorder with numerous co-occurring conditions (Himmelreich, Lutke, & Hargrove, 2020; Popova et al., 2016). Individuals with FASD have a wide range of diseases and disorders with a significantly higher incidence and earlier age of onset compared to age-matched individuals in the general population. Viewing FASD in this context shifts the narrow emphasis on brain and behavior to a broader one that encompasses the whole body and person. In addition, some of the behavioral and adaptive issues of those with FASD might be related to medical issues that are often undiagnosed (Choate & Badry, 2019; Himmelreich et al., 2020). These whole-body issues might also provide additional diagnostic approaches for identifying affected individuals, as well as differentiating them from individuals affected by other overlapping neurodevelopmental disorders. From the viewpoint of neuroethics, the development of inclusive and multidisciplinary training of clinicians who treat individuals with FASD, not only pediatricians but also family physicians who treat adults, is essential. A focus on how to provide an accurate diagnosis for this variable and often invisible disorder, and how to link patients to appropriate services and supports will go a long way toward supporting

and providing appropriate care for these individuals (Petryk, Siddiqui, Ekeh, & Pandey, 2019). Extending inclusive and multidisciplinary training to all professionals who work with individuals with FASD is also essential.

5. Ethical ramifications of biomarker use

Several social and ethical ramifications are emerging from the development and implementation of biomarkers for prenatal alcohol exposure and FASD. These include considerations related to the consequences of biomarker use in the context of stigma and consent, missed diagnoses, specificity and precision of the markers, information misuse, and differences between clinical and research applications. These differences can also manifest differently when discussing different types of biomarkers, such as those that focus on alcohol use or exposure versus child outcomes. However, these considerations ultimately link back to a single common theme—how can we ethically and responsibly use biomarkers in research and clinical settings to best serve the people they are ultimately designed to help?

5.1 Biomarkers of alcohol use or exposure

The real-time identification of prenatal alcohol exposure through biomarkers could help identify individuals who are drinking and thus enable or facilitate access to support and interventions to reduce drinking. However, this is an area fraught with problems of consent and stigma. As noted in Section 4, there is considerable stigmatization of pregnant individuals who may have substance use disorders, and the so-called blame for their children developing FASD can be misattributed as falling squarely on their shoulders. As such, many individuals, especially those who are pregnant, deny and hide their drinking due to the potential shame and blame associated with drinking. The same problem would likely occur with the implementation of real-time biomarkers of alcohol use in clinical settings, whereby consent to use these biomarkers would not be a given. Should consent not be provided, it remains unclear whether interventions or support could still be provided, or if the simple act of refusal could further stigmatize the individual and reduce the likelihood of seeking support at a later time.

Similar issues of consent and privacy would still arise if these maternal or child biomarkers were solely assessed in the child after birth, as the results would focus directly on the mother's actions and infringe on her rights, while leading to further stigmatization. In addition, not all children who

have prenatal alcohol exposure will necessarily develop any of the deficits or issues associated with FASD. As such, there is a potential to further stigmatize or discriminate against children themselves. Further, the application of these biomarkers would be fraught with potential issues of discrimination if they are not implemented as a standard screen for all births, as both conscious and unconscious biases could lead to healthcare providers targeting the use of these biomarkers to stigmatized vulnerable populations. The use of such approaches could disproportionately impact and further stigmatize vulnerable populations that experience higher rates of substance use and mental disorders, as well as those in lower socioeconomic areas that might be specifically targeted by care providers or governmental programs as high-risk. Thus, particular care must be taken when implementing biomarkers of alcohol exposure specifically, as these could have the potential to do further harm to vulnerable populations.

5.2 Biomarkers of child outcomes

By contrast, biomarkers that predict child outcomes have the potential to reduce the morbidity and possible mortality of diseases and disorders, as well as the adverse cognitive and behavioral effects of prenatal alcohol exposure by enabling early diagnosis and therefore early intervention. This is the crux of what is hoped for in biomarkers of FASD—that early diagnosis or early identification of risk will enable early intervention and support and thus reduce adverse outcomes over the life course. However, while early intervention might, indeed, support positive outcomes for the individual, using biomarkers to identify affected individuals might do nothing to decrease stigma at some levels, e.g., stigma by association or structural stigma, and might even increase stigma. Furthermore, as stigmatization increases blame and shame among both parents and children, it can push individuals and families away from seeking the treatments that can help improve their quality of life.

5.3 Missed or incorrect diagnoses

As noted in Section 3 on current biomarkers of prenatal alcohol exposure and FASD, there remain risks for false-positive or false-negative findings stemming from the use of biomarkers, as well as their specificity for different types of neurodevelopmental disorders. Thus, the missed or incorrect diagnosis of children with(out) FASD will be an important ethical concern moving forward.

Misattribution of FASD to typically developing children could have important ramifications for development. Beyond the issues surrounding stigmatization and discrimination described above, there is a possibility that incorrect diagnoses may lead children to believe that there is something wrong with them, which may lead to somatization or nocebo effects (i.e., harmful outcomes arising as a result of expectations of negative symptoms). Incorrect diagnoses may also lead to unnecessary, costly, and potentially intrusive treatments, which could have profound effects on child development. Similarly, as resources for FASD treatment and support remain limited, high rates of false positives could further reduce the availability of such resources to children and families who depend on them.

By contrast, false-negative findings from biomarkers could prevent access to those resources, which would increase the disparities and challenges facing children and families with undiagnosed FASD. Further, should deficits begin to emerge later in development, families may spend considerable time and resources attempting to obtain *any* diagnosis to help their child. In addition to the burden placed on families and children, these attempts could lead to further misdiagnoses and ensuing treatments that are poorly suited to children with FASD.

Similar to these issues around false-positive and false-negative diagnoses, the specificity of biomarkers must be rigorously assessed to prevent a missed etiologic diagnosis. Thus far, for most biomarkers of FASD, their specificity for FASD and/or extent to which they overlap with other neurodevelopmental disorders has not been investigated. Notable exceptions are the epigenetic signature of FASD, which could distinguish ASD from FASD cases (Lussier, Morin, et al., 2018), and eye tracking tools, which can currently differentiate between ADHD and FASD in research settings (Tseng et al., 2013). However, this initial step is a long way from establishing the specificity of these biomarkers for FASD as a potential etiology. As treatments differ among neurodevelopmental disorders (see Section 2.3), it will be crucial to assess the ability of biomarkers to distinguish between neurodevelopmental disorders. Nonetheless, biomarkers ultimately represent an opportunity to supplement symptom-based diagnoses with more empirical measures, which could increase the accuracy of neurodevelopmental disorder diagnoses and help better target interventions to those in need.

5.4 Clinical versus research-based biomarkers

Many, if not all, of the biomarkers discussed in the present chapter remain primarily research-based and have not yet been implemented in clinical

settings. However, the shift from research to clinical applications will need careful assessment and planning to prevent the issues of stigma, discrimination, and missed diagnosis described above. Further, many of the genome-wide tools have relied on population-level data, similar to studies of genetic risk using polygenic risk scores (PRS), where the risk of a disorder is relative to other people (Kumuthini et al., 2022; Lewis & Vassos, 2020). From this perspective, many of the biomarkers discussed in the present chapter and under development may reflect a measure of risk, rather than a distinct diagnosis of FASD or other neurodevelopmental disorder. As such, most biomarkers should be used in conjunction with other assessment tools to ensure reliable and accurate diagnoses.

The transition from research to clinical settings will also require careful consideration of implementation methods, which could occur through two distinct avenues or phases: (1) individual-level cases with a suspicion of exposure and (2) population-level screens. As discussed above, individual-level applications can be susceptible to bias and discrimination. However, this targeted approach could reduce the resources needed to implement biomarkers in clinical settings and thus, make them more readily accessible when needed. This approach could also ease the barriers to begin using biomarkers in the clinic, as their use would be more specific and likely to supplement other diagnostic methods. By contrast, population-level screens will require more deliberate and thorough assessments to ensure their robustness, as false-positive and false-negative diagnoses could lead to more widespread problems in these large-scale settings. For a more thorough discussion of the ethical considerations around population screening, see Spencer and Fullerton (2022).

6. Conclusions and next steps

Biomarkers for neurodevelopmental disorders represent an important next step in the development of better prediction and treatment strategies for children and families affected by neurodevelopmental disorders such as FASD. However, there remain several key steps and ethical considerations before they can be equitably and responsibly implemented in clinical and population-wide settings.

Beyond the issues discussed above, we should also consider who will use these biomarkers—one might suggest they be used for anticipatory guidance and surveillance regarding developmental, as well as physical and mental health outcomes. Yet, the data and studies to support this argument remain in their infancy and require further efforts to determine whether this strategy

would be effective at the individual and population-level. Further, we should also begin to consider how these biomarkers will be used, whether in the hospital for pregnant women, neonatal intensive care unit (NICU), or postnatally when there may be suspected neurodevelopmental disorders or ascertainment by child welfare services, and what the privacy and consent issues around their use will be. While specific categories of biomarkers may have different strategies for their use, we must carefully consider how their implementation will impact children and families who may or may not have neurodevelopmental disorders.

Ultimately, the ethical use of biomarkers for neurodevelopmental disorders will likely require extensive evaluation by ethics groups and potentially legislative action to ensure that all parties remain protected and receive the best care possible. Given the rapid evolution and emphasis on these biomarkers in research spaces, these next steps may happen sooner and with less diligence than they necessarily require. Nonetheless, the potential of biomarkers to improve the treatment and lives of people affected by neurodevelopmental disorders provides a strong incentive to further build and extend ethical frameworks for the development and implementation of biomarkers for these disorders.

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