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Dissecting FASD Through the Global Transcriptome

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Abstract

The recent study by Stepien, Lussier, Pavlidis, Kobor, and Weinberg demonstrates how prenatal alcohol exposure alters genomic expression far into the adulthood, and also provides a new view about how transcriptions might respond differently upon new environmental challenge. This study also provides a more comprehensive view by filling a gap of the global transcriptome analyses of FASD.

Keywords

Genomics; Gene-environment interaction; Epigenetics; Immune response; Microarray; Nutrition

It remains unanswered whether and how certain adverse environmental effects during pregnancy alter the developmental trajectory of the offspring, casting a temporary, long, and/or irreversible deficit into adult life. Drinking during pregnancy has been known to result in a wide range of outcomes including growth retardation, facial dysmorphology, brain undergrowth, and learning deficits collectively known as Fetal Alcohol Syndrome (FAS). More common (~10 times), however, are incidences in which the cardinal features of FAS (such as dysmorphic facial features) are not distinguishable and in which the range of the abnormalities vary— now categorically classified as Fetal Alcohol Spectrum Disorder (FASD). To date, the range and duration of the developmental disruption in FASD are really not well understood. This makes the diagnosis and treatment of the disease difficult, as reflected by the lack of comprehensive identification and efficient treatments to this day. It is important to ensure a better understanding of FASD as many abnormalities occurring in the early stages of life may be outgrown or delayed in manifestation. Assuming that phenotypes are the functional representations of the genome and epigenome, to better understand the holistic makeup of FASD, a series of global transcriptome analyses have been conducted to “profile” the alterations which might contribute to the dynamic dysfunctionality of prenatal alcohol exposure (PAE). The report from this paper entitled “Prenatal Alcohol Exposure Alters Steady-State and Activated Gene Expression in the Adult Rat Brain” by Stepien, Lussier, Pavlidis, Kobor, and Weinberg provided a new view to this pursuit but also demonstrated more surprises. This paper, through analysis of global gene expression changes in the hippocampus and frontal cortex, pointed out that PAE altered genes not observed earlier during development, and may lose “*adaptivity*”, such as normal

immune response to an environmental stressor in adulthood. This global analysis performed in adulthood also allowed for a comparison to early global gene expression analysis done previously (Da Lee et al., 2004, Hard et al., 2005, Shankar et al., 2006, Green et al., 2007, Downing et al., 2012, Rosenberg et al., 2010, Zhou et al., 2011c, Kleiber et al., 2012). The current studies filled the gap for longer term transcriptional effect (Kleiber et al., 2012). Together, these global transcription studies provided a collective view of the underpinnings behind the phenotypic abnormalities mentioned earlier. Also, a wider scope including various stages were presented through which a more complex picture was drawn (see below) in the depiction of FASD. As a note, this commentary is not aimed to re-analyze the multiple data sets, but to draw insights which are apparently not derived individually. I found at least three key features of the altered global transcription in FASD studies to be extraordinarily attractive — the transcriptome changes are transitional (and stage specific); the alterations are dynamic; and the alcohol does not act alone. They are detailed below.

Alcohol Induced Transcriptome Changes are Transitional and Stage Specific

Although many house-keeping genes are steadily expressed throughout life, many developmentally related genes are temporospatially expressed, peaking at certain periods of life or in particular tissues of organs. From the collective studies in **Table 1** (the Table is arranged in the order of stage of PAE and the age of analysis), it is found that the actively transcribed genes pertinent to the particular stage of development or tissue of active growth are highly sensitive and prone to the attack of PAE. At early stages, short PAE prior to neurulation at gestation day (E) 8 in mouse (Green et al., 2007) was found to affect genes of metabolic and cellular reprogramming and cytoskeletal formation. Specifically, significant up-regulation of genes involved in tight and adherens junction formation, focal adhesion, and regulation of the actin cytoskeleton were detected at the brink of changing *Wnt* signaling and apoptosis pathways. Affected expression networks also included MAPK1, IGF1, EGFR, PTEN and AKT1. These findings show that short exposure of alcohol (in hours) alters common signaling pathways and prompts cytoskeletal reorganization. Short PAE at the beginning of neurulation (E9), demonstrated by Downing et al (Downing et al., 2012), induced an altered expression of a subset of genes involved in apoptosis, cell cycle, zinc finger protein, methylation, chromatin remodeling, protein synthesis, and mRNA splicing.

PAE across key stages of neural tube formation (E8-E10) and axial and dorsoventral patterning (Zhou et al., 2011c) produced a collective reduction in expression of neural specification genes (*neurogenin*, *Sox5*, *Bhlhe22*), neural growth factor genes (*Igf1*, *Efemp1*, *Klf10* (*Tieg*), and *Edil3*), and alteration of genes involved in cell growth, apoptosis, histone variants, eye and heart development. There was also a reduction of retinol binding protein 1 (*Rbp1*), and *de novo* expression of aldehyde dehydrogenase 1B1 (*Aldh1B1*). Remarkably, four key hematopoiesis genes (glycophorin A, adducin 2, beta-2 microglobulin, and ceruloplasmin) were absent after alcohol treatment, and histone variant genes were reduced. This affected transcription profile is a highlight of early neurulation and hematopoiesis pertinent to this stage of development. At the stage of E6-8 PAE and analysis of

transcription at E10 or E15, Lee et al (Da Lee et al., 2004) found that genes related to craniofacial (palate and nasal epithelium) and lung formation were affected.

PAE at 1st-2nd gestation periods and analysis done near the end of second trimester equivalent discovered that apoptosis, proliferation, differentiation, regulation of growth factors, growth & remodeling, neuronal growth & survival in the brain tissue were affected (Hard et al., 2005). Additionally, apoptosis, proliferation, regulators of DNA transcription, glucose metabolism, lipid and fatty acid biosynthesis, as well as stress and stimuli response in the liver (Shankar et al., 2006) were affected. Relevant genes are highlighted in **Table 1**.

PAE throughout gestation and analysis of brain transcription at the young adult stage yields a wider view. These studies used mild or moderate alcohol exposure levels reaching blood alcohol levels (BAC) of 80-150 mg/dL (Stepien, 2014, Kleiber et al., 2012). Having their transcription analysis done in whole brain found subtle yet long-term alterations to brain gene expression that can be associated with FASD-related phenotypes. Some of these genes are known to contribute to neural system development and maintenance, with the disruption of their expression or function associated with cognitive phenotypes (**Table 1**) in mice and humans. These long-lasting transcriptome changes may underlie the life-long persistence of FASD-related cognitive and behavioral alterations. In the current issue, Kobor and Weinberg et al (Stepien, 2014) conducted their transcription analysis in hippocampus and prefrontal cortex and found PAE affect genes in metabolism, cell communication, development, transport, and signal transduction. Furthermore, PAE animals responded differently to an inflammatory challenge. The differential transcriptome responses are multifunctional, with roles in growth, proliferation, adhesion, structural organization, and cellular response to immunological or stressful stimuli. These differential responses are highlighted in ~2 week and subsided ~6 weeks after challenge. Interestingly, none of the genes found here directly overlapped with those previously identified in early gestation. These disparities, as proposed by the authors (Stepien, 2014), may be due to species- and sex-specific effects, differences between exposure paradigms, and different gene expression patterns in whole brain versus specific regions (Zhou et al., 2011c, Green et al., 2007, Hard et al., 2005). Moreover, the differentially responsive transcriptomes are transitional and are highly reflective of the time of PAE and the stage of analysis as indicated above. Their contributions to FASD might also be stage specific, but the consequences can be long lasting as development often relies on temporospatial coordination of gene expression and sequential cascades. Thus, it is not surprising that the phenotypic features of FASD are evolving post alcohol exposure, and may reach far into the young adult and perhaps life-long, while the altered gene transcription is transient and pertinent to the time and tissue of development. The profile of the differential global transcriptome per stage of PAE and stage of analysis would be valuable to understand in the transition of FASD.

Dynamic Responses to Life Challenges

A key study in this report (Stepien, 2014) demonstrated how global transcription of PAE rats were differentially altered in response to an environmental challenge in adult life. This is a broader definition of FASD gauged not by their baseline (which appear to be normal) but by their abnormal response (deficiency or aggravation) to insult. The study was extended from

their previous finding that PAE treated offspring has a tapered immune response upon challenge (Zhang et al., 2012). Up-regulation of immune-related genes normally occurs in the CNS in response to peripheral inflammatory stimuli. It is shown that two weeks after adjuvant challenge, PAE animals may fail to detect these immunological changes. The genes that responded to the challenge were different in PAE as compared to PF and Chow Controls rats—the *Mapk* and *Foxo*-related pathways, which are activated in signal transduction of the normal inflammatory cascade were suppressed. The *Adamts12* gene modulates neutrophil apoptosis during inflammation, while *Osm*, which attenuates the inflammatory response (Dumas et al., 2012, Cuadrado and Nebreda, 2010) was inhibited. These abnormal reactions may blunt their overall responses to adjuvant. Furthermore, the *Adcyap1* gene, modulating anti-inflammatory responses and acting as a neuroprotective agent in neurons following inflammation (Waschek, 2013), was tempered.

A number of other genes of functional importance, e.g. *Ghrhr* (growth hormone releasing hormone receptor), *Bhlhe40* (a basic helix-loop helix family gene), *Flna* (filamin A, alpha), *Ctgf* (connective tissue growth factor), *Vwf* (von Willebrand factor), and *Lcn2* (lipocalin 2), were also differentially responsive in PAE rats upon adjuvant challenge. These abnormal responses revealed a further environmental interaction with genes that were compromised above baseline regulation. One inviting explanation is that the epigenetic alteration regulating the transcription of these genes during PAE (which is under threshold) can be boosted by accumulating environmental insults later in life thereby altering the FASD transcriptome. For further reading see “Epigenetic medicine and fetal alcohol spectrum disorders” (Resendiz et al., 2013).

Contributing Factors Beyond Alcohol

Is alcohol alone the causality of the affected gene expression dynamics during PAE? Though the answer is not straight forward, a number of mitigating factors are being increasingly shown to play a role. Besides the co-use of other psychoactive substances, the nutrition, stress, and addictive state (e.g. withdraw) are adherent cofactors, frequently coinciding with alcohol intake to impact gene transcription. Current animal studies provide a salient view of these factors by analyzing a pair-fed (PF) group that is matched at the nutrition level of the alcohol group. Their study showed that common transcriptional change in the brain occurred in both PF and Alcohol groups, including neurotrophic factor related genes e.g. *Igf2* (insulin-like growth factor binding protein 7), neural receptor genes e.g. *Grik5* (glutamate receptor, ionotropic, kainate 5) and *Gabbr2* (gamma-aminobutyric acid GABA A receptor, rho 2), homeodomain genes, e.g. *LOC301193* (similar to Discs large homolog 5), cell adhesion genes e.g. *Nrxn3* (neurexin 3), and metabolic genes e.g. *Atp5a1* (an ATP synthase) and *Acs11* (acyl-CoA synthetase long-chain family member) (Stepien, 2014). Some of the genes responses are widely different among Alcohol, PF, and Chow Controls, [e.g. *Igf2* (insulin-like growth factor 2), *Col8a1* (collagen, type VIII, alpha 1), and *Hbb-b1* (hemoglobin, beta adult major chain)]. On the other hand, a report by Downing et al. indicated that very few genes were differentially expressed between maltose-exposed PF and Chow groups (Downing et al., 2012). Perhaps the difference lies in that Downing et al's study used a short period of treatment with gavage administration for 4 hrs (see Table 1), leaving little time for protracted responses. In summary, the nutritional effect collaborating

with alcohol's impairment is confirmed in a defined under-nutrition study in conjunction with alcohol treatment. In this study a large scale of transcriptional abnormality was found, including a highly differentially affected growth related gene *Igf1* (Shankar et al., 2006).

In the Kobor and Weinberg et al study (Stepien, 2014), there are also genes differentially altered in PF in comparison to either alcohol or Chow group (listed in Table 7 of their report). These gene alterations are unique in their own right, since they cannot be categorized into nutritional disparity or other effects of alcohol. It is however known in the field that the PF group is under a stress condition in that they consume the majority of their food quotas in the early hours, depleting food for the remainder of the day when hunger may still occur. If this factor plays a role, the stress of hunger may contribute to the differences in gene transcription that are unique to the PF condition.

Future Studies

The stage-wide transcriptome analysis is useful to understand the concurrent phenotypic features of FASD. It is also a helpful reference to understand the history during the timing of PAE when assessing teratogenesis at later stages. As indicated above, the alcohol-transcriptome can be affected by dose, timing, and length of the PAE. It also varies with animal strain, gender, tissue, and time of analyses. This area of research would be more fruitful with coordinated and team efforts. Two additional aspects derived from the study in this issue and from others are ---the nutritional factor is likely a collaborative culprit exacerbating the not only in phenotypes FASD (May and Gossage, 2011, Fuglestad et al., 2013) but also in transcriptomes. Future studies on FASD treatment and mechanism may take an alternative approach to investigate whether enriched nutrition may ameliorate the severity (Thomas et al., 2010; Wozniak et al., 2013; May et al., 2014) and abnormal transcriptome of FASD. Second, the ability of responses to life challenges in FASD is a less documented area that needs more study. Besides a better understanding of the FASD transcriptome for design of treatment in the future, transcriptome analysis can also serve as biomarker for examination against surrogate tissue. A placenta was studied under this notion (Rosenberg et al., 2010).

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Table 1

Differential alteration of transcriptomes among different paradigms.

Animal	Blood alcohol (mg/dL)	PAE	Age of Analyses	Tissue	Example of Key Genes	Feature	Reference
B6	~370 (estimate)	E8-E8+3h	E8+3h	Embryos	<i>MAPK1, Aldh3A2, CD14, Pfkfb, Tnfrsf1A, RPS6, Igfl, Egfr, PTEN</i> and for <i>PK11195 at AKT1</i>	Metabolic and cellular reprogramming	(Green et al., 2007)
B6 (D2)	Peak>310	E9-E9+4h	E9+4h	Head	<i>Igf, Bcl3, Brms1l, Foxc1, Suv420h1, Zfp</i>	Apoptosis, cell cycle, methylation, chromatin remodeling, mRNA splicing	(Downing et al., 2012)
B6	100-350	E8-10	E10	Embryo	<i>Neurogenin Sox5, Bhlhe22, Igfl, Efemp1, Klf10, glycophorin A, adducin 2, ceruloplasmin</i>	Genes related to neurulation and hemopoiesis	(Zhou et al., 2011c)
B6		E6-8	E10 or 15	Embryos	<i>Plunc, neurofilament, Zinc finger, neuregulin4, Crystallin, Heat-shock protein, Annexin A1.</i>	Genes related to palate, lung, and nasal epithelium	(Da Lee et al., 2004)
B6	400	E7 and E9	E18	Brain	<i>Timp4, Bmp15, Rnf25, Akt1, Tulp4, Dexas1</i>	Apoptosis proliferation, differentiation, regulation of growth factors, growth & remodeling, Neuronal growth & survival	(Hard et al., 2005)
S-P	~250-350	E5-15	E20	Liver	<i>TGFB, Igfl, Ghr, Akt1, Cdc37, Fabp5, Eno1, scd2, FASN, Gclm, Gstm2, Ube2d3</i>	Apoptosis, proliferation, Regulators of DNA transcription, Glucose metabolism, Lipid and fatty acid biosynthesis, Stress and stimuli response	(Shankar et al., 2006)
B6	Assume 80-120	E0-P10	P70	Whole brain	<i>Ache, Bcl2, Cul4b, Dkc1, Ebp, Lcat, Nsdh1, Sstr3, Nsdh1, Bcl2, Otx2, Sstr3</i>	Cellular and tissue development, free radical scavenging, and small molecule metabolism, and neurobehavioural phenotypes	(Kleiber et al., 2012)

Animal	Blood alcohol (mg/dL)	PAE	Age of Analyses	Tissue	Example of Key Genes	Feature	Reference
S-P	100-150	E1-21	~10-14wks	Brain region (Hipp, PFC)	<i>Tcf4, Pex11g, Ndfip1, Acsl3, Dusp6, Atp6ap1, Atp6ap1, Caap1, Cnih2.</i> Response to adjuvant: <i>Ghrhr, Flna, Sgk1, Vwf, Lcn2, Ctgf</i>	Metabolism, cell communication, development, transport, and signal transduction.	(Stepien, 2014)

Note: Hipp: hippocampus, PFC: prefrontal cortex; B6: C57/BL6 mice; (D2): DAB2 mice with minimal transcription alteration. S-P: Sprague-Dawley rat.

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