Supplementary Discussion

In support and expansion of Table 1, the ensuing text provides a detailed description of the relevance to NDD-related and MS-associated phenotypes of a subset of annotations mapped to DEGs shared among DRD1-expressing striatal cells of first- and second-generation DNE mice.

Shared Neurobehavioral Deficits

At a macroscopic level, the overrepresented *behavior*, *regulation of behavior*, *motor behavior*, and *positive regulation of circadian rhythm* annotations of shared DEGs are broadly consistent with NDD-related behavioral phenotypes such as hyperactivity, inattention, impulsivity, sleep and circadian rhythm disruptions, and predispositions to smoking in the children of maternal smokers, with the findings of ours and others' prior animal model studies that DNE precipitates intergenerational transmission of NDD-like hyperactivity, risk-taking behaviors, aberrant rhythmicity of activity, and increased nicotine consumption and preference, and with analogous neurobehavioral deficits such as hyperactivity, impulsivity, sleep disruptions, aberrant rest-activity activity rhythms, and affinity for smoking which are transdiagnostic features of NDDs including ADHD, autism, and schizophrenia [9, 14, 20-24, 50-55, 150-153].

Shared Developmental Encephalopathy + Neuroteratogenicity

The overrepresented *brain development*, *central nervous system myelination*, and *neuron development* annotations of shared DEGs are commensurate with the impairments of brain and neural development and myelination in the children of maternal smokers, with the aberrant neuronal differentiation, maturation, and migration alongside reduced cortical volume and thickness in animal models thereof, and with the developmental encephalopathies such as decreased total brain volume, reduced cortical volume and thickness, and impaired neuronal differentiation which are transdiagnostic features of NDDs [25-29, 52, 57-59]. Similarly, the overrepresented *detoxification*, *neuron cellular homeostasis*, and *negative regulation of neuron death* annotations parallel the increased neuronal morbidity and apoptosis and reduced neuronal density and activity reported in the children of maternal smokers, the atypical neuronal morphology and apoptosis as well as the abovementioned perturbed neuronal differentiation, maturation, and migration coupled with reduced cortical volume and thickness in animal models thereof, and the markers of neuroteratogenicity such as the aforementioned decreased total brain volume, reduced cortical volume and thickness, and impaired neuronal differentiation that are transdiagnostic features of NDDs [25-30, 52, 57-59].

Shared Neurotransmitter System Dysfunction

From a neurobiological perspective, the overrepresented *acetylcholine neurotransmitter release cycle*, *regulation of synaptic transmission*, *dopaminergic*, and *dopaminergic neuron differentiation* annotations of shared DEGs are reminiscent of atypical nAChR expression patterns and irregularities in DA receptor and reuptake systems in the children of maternal smokers, of multigenerational perturbations in nAChR expression and function, DA neurotransmission, and DAT function documented in first- and second-generation DNE animal models, and of neurotransmitter system dysfunctions such as polymorphisms in and aberrant expression and function of nAChR, DA receptor, and DAT subtypes which constitute transdiagnostic features of NDDs including ADHD, autism, and schizophrenia [31-33, 52, 54, 56-57, 60-63, 66-68].

Shared Neurotrophic Dysfunction

The overrepresented *neuropeptide signaling pathway*, *signaling receptor ligand precursor processing*, and *trans-synaptic signaling by BDNF* annotations of shared DEGs are consonant with the abnormal BDNF concentrations in umbilical cord sera and the aberration of BDNF-mediated phenotypes including neuronal differentiation and development, learning and memory, and synaptic plasticity in the children of maternal smokers, with the distortion of mesocorticolimbic BDNF signaling in first-generation DNE rodents which appears to extend to at least the frontal cortices and striata of second-generation DNE progeny, and with markers of neurotrophic dysfunction such as disruptions of neurodevelopment, synaptic genesis, plasticity and pruning, and learning and memory, which are transdiagnostic features of NDDs [34-36, 64-72, 154].

Shared HPA Axis Dysregulation

The overrepresented *stress response*, *intracellular steroid hormone receptor signaling pathway*, and *regulation of hormone secretion* annotations of shared DEGs are akin to markers of HPA axis deregulation in the children of maternal smokers including hypocortisolemia, aberrant glucocorticoid receptor expression, and altered stress responsivity, to our previous findings of DNE-evoked intergenerational hypocorticosteronemia and corticostriatal GR hypoactivity, and to the HPA axis dysregulation such as hypocortisolemia and altered GR expression which are transdiagnostic features of NDDs [37-39, 72].

Shared Neuroimmune and Neuroinflammatory Aberrations

The overrepresented *annexin, inflammatory response*, *MHC I, regulation of I-kappaB kinase/NF-kappaB signaling*, *response to interleukin-4, neutrophil degranulation, glutathione synthesis and recycling, response to oxidative stress*, and *HSF1 activation* annotations of shared DEGs concur with indicators of neuroimmune and neuroinflammatory aberrations including aberrant toll-like receptor (TLR)-mediated immune responsivity, increased microglial markers and microglial

polarization, altered cytotoxic and regulatory T-cell counts, and atypical levels of inflammatory markers such as interleukins and interferons in the children of maternal smokers and animal models thereof along with the hyperactivity of innate and adaptive immune systems, aberrant crosstalk between neurons and glia, and overconcentration of pro-inflammatory factors such as cytokines, chemokines, and eicosanoids observed in NDDs and encompassed by the neuroimmune and neuroinflammatory aberrations transdiagnostic feature thereof [43-47].

Shared Epigenomic Perturbations

Potentially acting as a mechanistic hub for the neurotranslatomic perturbations and by extension the NDD-like brain and behavioral phenotypes encapsulated by the abovementioned annotations, the overrepresented *positive regulation of histone deacetylation*, *PRC2 methylates histones and DNA*, and *chromatin-mediated maintenance of transcription* annotations of shared DEGs are congruent with anomalous epigenotypes including altered global and gene-specific DNA methylation profiles, histone modifier expression and histone modification patterns, and nucleosome/chromatin architectures documented in the children and (for DNA methylome perturbations only) the grandchildren of maternal smokers and implicated in the reprogramming of gene expression therein, with ours and others' prior findings of corticostriatal global DNA methylomic, histonomic, DNMT3A, MeCP2, and HDAC2 deficits in first- and second-generation DNE animal models, and with the epigenomic perturbations such as DNA methylomic, histonomic, and chromatinomic alterations which are transdiagnostic features of NDDs including ADHD, autism, and schizophrenia [40-42, 73, 75-77, 81-88, 150, 155-165]. The overrepresented *spermidine biosynthesis* and *S-adenosylmethionine* (SAM) *metabolic process* annotations further exemplify epigenomic perturbations that are transdiagnostic feature of NDDs, insofar as SAM is a precursor (methyl-donor) for the biosynthesis of polyamines such as spermidines (and also for the methylation of DNA) which themselves are pan-inhibitors of both the expression and the activity/function of multiple DNA methyltransferases (all of which utilize SAM as a methyl-donor for the methylation of DNA) [76, 166]. Interestingly, this information further suggests that the DNE-induced intergenerational DNMT3A deficits and corresponding global DNA hypomethylation detailed in our prior works may be orchestrated in part by atypification of polyamine homeostasis.

Specific DEGs unique to F1 NIC or F2 NIC mice

Many interesting DEGs were identified that are unique to DRD1-expressing striatal cells of either first- or second-generation DNE offspring. Therein, notable DEGs uniquely detected in DRD1-expressing striatal cells of first-generation DNE mice include *Polr2f, H2ax,* and *Cops6.* Therein, the

differential expression of *Polr2f*, which encodes an RNA polymerase II subunit, is broadly congruent with the generalized dysregulation of gene expression thought to mediate the etiologies of ADHD, autism, and schizophrenia [145, 147-149]. Moreover, the differential expression of *H2ax*, which encodes an H2A-family histone, is compatible with previous studies implicating histonomic anomalies in the pathophysiology of NDDs including ADHD, autism, and schizophrenia [150, 174-176]. Lastly, the differential expression of *Cops6*, which encodes one of eight COP9 signalosome subunits, is notable in light of the indispensable and evolutionarily conserved roles of the COP9 signalosome in NDD-related processes such as neurodevelopment and circadian rhythms as well as the COP9 signalosome abnormalities associated with NDDs including autism and schizophrenia [177-179].

On the other hand, notable DEGs uniquely detected in DRD1-expressing striatal cells of second-generation DNE mice include *Snrpf, Hsp90aa1,* and *Mybbp1a.* Firstly, the differential expression of *Snrpf*, a small nuclear ribonucleoprotein polypeptide is congruent with the fundamental roles of small nuclear ribonucleoprotein polypeptides in development alongside their reported deregulation in autism [180]. In addition, the differential expression of *Hsp90aa1*, an inducible form of HSP90 that reacts to cytostress and in part regulates DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) is material to the implications of heat shock proteins in neurodevelopmental regulation, in the etiologies of NDDs, and in the induction by DNE of intergenerational MeCP2 and HDAC2 deficiencies evinced by our prior work [76, 181]. Finally, the differential expression of *Mybbp1a*, which encodes the predominately nucleolar transcriptional regulator MYBBP1A is salient considering the essential function of MYBBP1A in embryonic development as well as its circadian regulatory role as a corepressor of *Per2* expression [182-183].

Word frequency analysis of DEGs shared among or unique to F1 NIC and/or F2 NIC mice

Word frequency analyses of DEG-encoded protein annotations yielded macroscopic insights into and revealed broad biological themes characteristic of the enriched and depleted differential translatomes in DRD1-expressing striatal cells of first- and second-generation DNE mice as well as those shared therebetween. For the enriched differential translatome shared between first- and second-generation DNE mice, the frequent occurrences of words such as *apoptosis* and *vesicles* within DEG-encoded protein annotations are congruent with transdiagnostic features of NDDs including neuroteratogenicity and neurotransmitter system dysfunction, respectively, while the frequent occurrence of the word *ceramides* within shared enriched DEG-encoded protein annotations concurs with the broad metabolic abnormalities (including altered ceramide concentrations) documented among ADHD, autism, and schizophrenia patients [129]. For the depleted differential translatome shared among first- and second-generation DNE mice, the frequent occurrences of

words such as *development*, *hormone*, and *glutamine* within DEG-encoded protein annotations are emblematic of transdiagnostic features of NDDs including developmental encephalopathy, HPA axis dysregulation, and neurotransmitter system dysfunction, respectively.

For the enriched as well as depleted differential translatomes detected in first-generation DNE offspring, the frequent occurrences of words such as *damage*, *degradation* and *trafficking* as well as *chromatin*, *histones*, and *calcium*, respectively, within DEG-encoded protein annotations are consistent with transdiagnostic features of NDDs including developmental encephalopathy, neuroteratogenicity, neurotransmitter system dysfunction, and epigenomic perturbations.

For the enriched differential translatome detected in DNE grandoffspring, the frequent occurrences of words such as *transmembrane*, *stress*, and *metabolism* within DEG-encoded protein annotations are consistent with transdiagnostic features of NDDs including neurotransmitter system dysfunction and HPA axis dysregulation and with the growing bodies of evidence implicating metabolic and mitochondrial dysfunctions in NDDs, respectively [128]. For the depleted differential translatome detected in DNE grandprogeny, the frequent occurrences of words such as *differentiation*, *growth*, and *histone* within DEG-encoded protein annotations are compatible with transdiagnostic features of NDDs including developmental encephalopathy, neurotrophic dysfunction, and epigenomic perturbations, respectively.

Specific MS-associated DMGs orthologous to DNE-induced DEGs unique to F1 NIC or F2 NIC mice

For F1 NIC mice alone, DNE-dependent DEGs orthologous to MS-associated DMGs included *PBX2*, *KCNH3*, *LEFTY1*, *DDIT4, and DTNBP1*, while those for F2 NIC mice alone included *CRELD2*, *HSPB1*, *CREB3L2*, *H1F0*, and *LRRK2*. Within the F1 NIC vs. PACE intersection, *PBX2* is noteworthy for its mediation of brain and neuron development and its association with autism and schizophrenia, *KCNH3* attracts attention given its influences on NDD-related phenotypes such as inattention and impulsivity and its implication in ADHD, *LEFTY1* is prominent based on its role in embryonic development and morphogenesis, its transcriptional regulation via DNMT3A-mediated DNA methylation, and its implication in schizophrenia, *DDIT4* (which encodes REDD1) stands out for its relation to neuronal genesis, differentiation, migration, development, inflammation, and death and its association with autism and schizophrenia, and *DTNBP1* is striking due to its roles in neuronal survival and viability, synaptogenesis, and DA and glutamate neurotransmission as well as its linkage to schizophrenia [184-195].

For the F2 NIC vs. PACE intersection, *CRELD2* is salient because it regulates α4β2 nAChR expression and is implicated in ADHD, autism, and schizophrenia, *HSPB1* stands out owing to its involvement in neuronal development and maturation, neuroinflammation, and neurodegeneration as

well as its differential expression in autism, *CREB3L2* is pertinent since it modulates NGF-evoked neurodifferentiation, axon growth, and sonic hedgehog signaling and is associated with ADHD, autism, and schizophrenia, *H1F0* (a linker histone) is important for contribution to chromatin and nucleosome architecture, brain and neuronal development, and autism and schizophrenia risk, and *LRRK2* garners attention for its role in axonal and neuronal development in the midbrain DA system alongside its implication in autism and Parkinson's disease [196-197].

Annotations of MS-linked DMGs orthologous to DNE-induced DEGs unique to F1 NIC or F2 NIC mice

Annotations mapped to trans-omic orthologs unique to the F1 NIC vs. PACE or F2 NIC vs. PACE intersections are emblematic of several transdiagnostic features of NDDs. Therein, the overrepresented *general adaptation syndrome*, *regulation of corticosterone secretion*, *regulation of dopamine receptor signaling pathway*, *neurotrophin signaling pathway*, *negative regulation of nervous system development, positive regulation of acute inflammatory response*, *positive regulation of immune system process*, and *astrocyte cell migration* annotations mapped to the F1 NIC vs. PACE intersection span transdiagnostic features of NDDs including HPA axis dysregulation, neurotransmitter system dysfunction, neurotrophic dysfunction, neuroimmune and neuroinflammatory aberrations, and developmental encephalopathy and concur with the HPA axis, DAergic, and DAT dysfunction as well as the (pro)BDNF imbalance linked to MS and shown to undergo intergenerational transmission in animal models thereof, while the overrepresented *positive regulation of DNA demethylation*, *regulation of histone deacetylase activity*, *regulation of dopamine receptor signaling pathway*, *positive regulation of neuroinflammatory response*, *striatum development, regulation of cytokine production*, *innate immune response*, and *regulation of microglial cell activation* annotations mapped to the F2 NIC vs. PACE intersection align with transdiagnostic features of NDDs including developmental encephalopathy, neuroteratogenicity, neurotransmitter system dysfunction, neuroimmune and neuroinflammatory aberrations, and epigenomic perturbations and are tantamount to phenotypes linked to MS and/or exhibiting intergenerational transmission in DNE animal models, such as atypical global and gene-specific DNA methylation profiles, histone modifier expression and histone modification patterns, nucleosome and chromatin architectures, and DNMT3A and MeCP2 levels alongside neuroinflammation, striatal hyperdopaminergia, and DAT dysfunction.

Perturbagen classes connected to DEGs unique to F1 NIC or F2 NIC mice

Results from analysis of pharmacologic perturbagen classes revealed that *PI3K inhibitor* (-) and *MTOR inhibitor* (-) were connected to DEGs unique to F1 NIC mice, while *HSP inhibitor* (+) and *proteasome inhibitor* (+) were connected to DEGs unique to F2 NIC mice. These results are a microcosm of the wide-ranging and far-reaching alterations of fundamental cellular process elicited by

DNE and MS and support the growing recognition of NDDs as highly multi-faceted conditions from both mechanistic and diagnostic perspectives.

Individual perturbagens connected to DEGs unique to F1 NIC or F2 NIC mice

Descriptions of noteworthy individual pharmacologic perturbagens connected to DEGs unique to F1 NIC mice included *glutamate inhibitor* (-), *acetylcholinesterase inhibitor* (+), and *glucocorticoid receptor agonist* (+), while those connected to DEGs unique to F2 NIC mice included *dopamine receptor agonist* (+), *calcium channel blocker* (-), and *cytochrome P450 inhibitor* (+). These findings are consistent with phenotypes such as aberrant DA, ACh, and GC signaling which underpin neurotransmitter system dysfunction and HPA axis dysregulation as transdiagnostic features of NDDs, as consequences of MS, and as intergenerational impacts of DNE in animal models.