Transgenerational latent early-life associated regulation unites environment and genetics across generations

The origin of idiopathic diseases is still poorly understood. The latent early-life associated regulation (LEARn) model unites environmental exposures and gene expression while providing a mechanistic underpinning for later-occurring disorders. We propose that this process can occur across generations via transgenerational LEARN (tLEARn). In tLEARn, each person is a 'unit' accumulating preclinical or subclinical 'hits' as in the original LEARN model. These changes can then be epigenomically passed along to offspring. Transgenerational accumulation of 'hits' determines a sporadic disease state. Few significant transgenerational hits would accompany conception or gestation of most people, but these may suffice to 'prime' someone to respond to later-life hits. Hits need not produce symptoms or microphenotypes to have a transgenerational effect. Testing tLEARn requires longitudinal approaches. A recently proposed longitudinal epigenome/envirome-wide association study would unite genetic sequence, epigenomic markers, environmental exposures, patient personal history taken at multiple time points and family history.

First draft submitted: 6 October 2015; Accepted for publication: 11 December 2015; Published online: 7 March 2016

Keywords: aging • childhood • development • epigenetics • experiences • insult • intergenerational • late life • neurodegenerative • nutrition • post traumatic

Background

Recent human genome research, accompanied by ever-increasing technological developments, continues to significantly enhance our understanding of human diseases in terms of etiology, diagnosis, drug response and novel treatment avenues. Diseases are often heterogeneous (several diseases overlapping as one) and multigenic, meaning disease phenotype (symptoms) can be caused by multiple genes/mutations, although each individual might or might not have one main independently causal rare variant. Exclusive use of homogeneous populations (e.g., Canadian founder, Icelandic and Utah cohorts) in studies will not yield clinically relevant biomarkers applicable to the population at large.

In the present Special Report, we will briefly mention the advances and limits

of current genetic research and technology and then focus onto gene–environment interaction that would support and expand various genetic models to explain human diseases. Our premise is that sporadic cases of Alzheimer's disease (AD), Parkinson's disease (PD), suicide and other disorders display well-established environmental associations. The latent early-life associated regulation (LEARn) model integrates early-life events, environmental exposures and gene expression while providing a mechanism for disorders occurring later in the life of an organism. Here, we propose that this process can also occur across generations via transgenerational LEARN (tLEARn). We provide evidence from different research studies in favor of the proposed concept, tLEARn, which uniquely interconnects environmental

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influence upon genes via epigenetic modification due to early-life ‘hits’, with the epigenomic record of such ‘hits’ being passed along to further generations.

Genomic variations: small variants, copy number variants & rare variants do not add up to explain the cases of sporadic diseases

Human genomes are usually considered a spectrum of ‘normal’ variation within an ethnically diverse population. A small fraction of variants can be identified, which may be unique to a disease or drug profile. Personalized medicine would be based on defining genetic subgroups by rare genetic variants. According to the common variant hypothesis, which postulates that gene variation within the human founding population underlies the predisposition to heritable diseases, most SNPs have little clinical value.

At best, most SNPs discovered in genome-wide association studies (GWAS) associate with slight increases in disease incidence and are often described as ‘predisposition’, ‘risk’ or ‘susceptibility’ factors. The research value of these changes points toward biochemical pathways of interest, particularly to discover intervention targets that are perturbed by environmental factors under the reasonable presumption that a genetic risk mimics the effect of environmental damage upon that gene’s normal expression. This has been the case in the use of statins in response to genetic discoveries regarding HMG reductase to reduce low-density lipoprotein cholesterol [1]. Furthermore, cumulative effects of small genetic changes may produce great overall variability in psychiatric conditions like bipolar disorder [2].

Genome-wide biomarker discovery platforms systematically reveal rare genetic variants that could be used in combination to differentiate responders from nonresponders and to predict serious adverse events in drug development trials by shared biochemical complexes in which these genes participate. Furthermore, while copy number variants (CNVs) and other structural variants make a major contribution to genetic variation, they are frequently found in both healthy people and those with sporadic diseases, such as autism spectrum disorder (ASD), schizophrenia and PD. Recently, the importance of *de novo* (occurring for the first time in an individual) mutation in disease etiology has been recognized [3,4]. Rare genetic variants have recently been found to play a larger role in diseases than previously known – up to 7% of cases of bipolar disorder, schizophrenia or ASD [5]. This could potentially be of aid to millions of people, depending on overall disease prevalence. On the other hand, a model that explains up to 7% of cases still does not explain over 90% of cases, and in some situations, such as ASD with IQ ≥100, *de novo* mutation has no correlation [3].

Genetic models can be greatly supported and expanded by explicit mechanistic explanations of gene–environment interaction. For example, well-established environmental associations have been found for PD [6], AD [7,8], suicide [9] and other disorders [10,11]. A model is needed that incorporate the role of environment, including both early and later-life exposures, the resultant ‘somatic epitypes’, their effects on adult-life disorders and potentially beyond a single lifespan. Such a model would provide a unique concept, tLEARn – the major focus of the present Special Report. This concept will interconnect environmental influence upon genes via epigenetic modification due to early-life hits with the epigenomic record of such hits being passed along to further generations.

Genetic variation on its own does not explain all pathogenesis of ‘sporadic’ disorders

The origin, pathogenesis and trajectory of idiopathic or sporadic diseases, including some forms of neurodegenerative disorders, that do not follow the Mendelian law of inheritance have attracted increasing attention in recent years, particularly in developing models of polygenic inheritance, endophenotypes, *de novo* mutation and others. Large-scale genomic association studies have pointed toward pathway disruptions that could occur at any point, not just single gene [12]. Nevertheless, in some disorders with known genetic components, such as AD, cases that can be explained solely by genetic mutation are a minor fraction. Those forms of AD that are autosomally inherited and traceable to specific DNA variations account for no more than 10% of cases [13]. Primary DNA sequence variation certainly accounts for substantial proportions of variance for some diseases, but environment and gene × environment interaction can account for a greater proportion in others. Epigenetics would be no panacea. The epigenetic approach would be an additional tool to be used when appropriate for a specific disorder.

There is an important role played by epigenomic factors in conditions as diverse as AD [14–16], PD [16–18], ASD, schizophrenia and even suicide [19–21]. The field has determined epigenetic changes for specific genes, such as *SHANK3* [22], brain region-specific epigenetic alterations [23] and several differences in the epigenome between monozygotic (MZ) twins discordant for ASD [24]. This epigenomic discordance is of particular interest because there is not a simple epigenome-wide difference between ASD and non-ASD MZ twins. Instead, differential methylation was clustered around specific loci, including *NLGN2*, *SNRPN* and ATP-sensitive inward rectifier potassium channel 10 (*KCNJ10*), among others. In addition, further site-specific methy-

lome differences were found to depend upon whether ASD was familial or sporadic [25–27]. In AD, the epigenomic evidence is as direct as brain cortical neuron-specific differences in gene promotor methylation in twins discordant for AD [28] and overall hypomethylation and hypohydroxymethylation of DNA from hippocampus of AD patients versus non-AD subjects [29]. However, epigenetic effects on neuropsychiatric issues are not simply a matter of insufficient DNA methylation. In fact, brains from victims of suicides show increased methylation [19,21]. Bipolar disorder is also associated with increased DNA methylation and gene repression, including *KCNQ3* [30–32]. *BDNF* was subject to hypermethylation and downregulation in major depressive disorder [33,34].

However, DNA modification is not the sole form of epigenomic marker. The other major epigenomic marker is the modification of histones, including acetylation, phosphorylation and ubiquitination, among others [35]. Aberrant histone acetylation is associated with AD [14] and post-traumatic stress disorder (PTSD) [7]. Exposure to the pesticides paraquat and dieldrin are associated with PD [36]. Dieldrin and other pesticides also induce hyperacetylation of histones H3 and/or H4 [37,38], and such induced hyperacetylation is strongly associated with dopaminergic neurotoxicity [6,39]. Of particular interest, psychiatric (antidepressant) effects have been noted for epigenetic drugs, particularly the histone deacetylase inhibitor MS-275 [40]. In addition, nicotine, heroin, methamphetamine, cocaine and ethanol each induce chromatin-altering modifications of histones when administered through methods typical of their abuse. These alterations are followed by changes in multiple pathways in brain reward circuitry [41].

Epigenomic contributions to neurobiological disorders may actually reach across generations. For example, an association has been found between paternal age at conception and risk for ASD and schizophrenia in offspring [42–44]. While one interpretation has been an accumulation of germ-line DNA mutations, an alternate mechanism would be an accumulation of environmentally induced epigenomic changes. The connection between environmental stress and epigenomic changes is well known [45–47]. The idea that prenatal and perinatal conditions influence health in childhood and later life has been long established. That the life of the father may also exert an influence adds another level of complexity.

A molecule that has been getting a good deal of attention in the literature is miRNA, which is short, noncoding RNA capable of modulating protein expression by acting as a set of specific recognition site ‘sockets’ in the RISC translation regulation

complex, primarily targeting the 3’UTR of associated mRNA [48]. Modulation of various epigenetic markers requires miRNA activity in *Arabidopsis* [49] and has been shown to exist in mouse models [50] and clinical samples [51]. miRNA-mediated methylation [49,52–54], acetylation [50] and ribosylation [55] have been reported in the literature – primarily related to tumorigenesis. Thus, while miRNA plays ‘a role’ in epigenetics, such a role would be, therefore, analogous to *DNMT* or *HDAC* or proteins that regulate *DNMT* or *HDAC* and does not grant miRNA a special status as an epigenetic agent. Environmental factors such as cigarette smoke [56], physical activity [57], metal exposure [58] and infection [59] can regulate miRNA levels. The miRNA genes can be under the influence of epigenetic modification [60–62]. We do not claim that miRNA is specifically inherited as an epigenetic marker or any sort of particularly epigenetic factor, any more than any other DNA transcription product.

LEARn unites environmental exposures & gene expression while providing a mechanistic underpinning for later-occurring disorders

The LEARN model encompasses environment–genome interaction in the etiology of sporadic neuropsychiatric disorders. Such disorders include, but are not limited to ASD, affective disorders, neurodegenerative disorders such as AD and PD, and others. The model connects environmental influence to biological outcomes through the mechanism of altering biochemical (such as epigenetic) markers. In short, LEARN posits that, for many sporadic disorders, the body accumulates ‘hits’ (that may include some genetic predispositions), but no single hit is sufficient to cause disease, instead, each is individually dormant. Many hits are of environmental origin and are ‘recorded’ and maintained in the organism through epigenomic markers (Figure 1). If a sufficient number of ‘correct’ hits occur before a critical developmental/aging threshold, the organism will develop the corresponding disorder [63,64]. The critical elements of the LEARN model are latency and that the latent hits can affect the organism even if the specific (causative) stimulus has long since been cleared, since the immediate biochemical effect is a persistent epigenomic alteration. LEARN-mediated disorders are not immediate acute products of a malign stimulus ASD, AD, PD or others would not appear until a critical threshold of hits is reached, and this critical threshold may include normal biological stages of development, maturation and aging. This is similar to the ‘n-hit’ model of oncology [63,65].

Figure 1. The latent early-life associated regulation model. LEARN explains idiopathic disorders on the basis of accumulation of 'hits' through an organism's lifespan. Hits can be environmental, genetic or epigenetic. **(A)** Genetic pathway: a 'purely' genetic disorder, such as Alzheimer's disease (AD) due to APP KM670/671NL (Swedish) mutation. AD-associated genes' DNA sequence variant determines disease state in an autosomal dominant fashion ('familial' AD/FAD). **(B)** Development of disease via LEARN pathway. A primary 'hit' between conception and a critical developmental threshold instills a latent epigenetic change. If this is followed by subsequent 'hits' later in life, accumulated risk factor effects reach clinical disease state as in the sporadic AD. **(C)** LEARN pathway across generations: tLEARN. Similar to LEARN progression except that the original 'hit' has occurred in an earlier generation and been transmitted asymptotically by epigenetic inheritance. **(D)** Remediation pathway or 'Averted' LEARN. Given that many epigenetic marker states can be altered by environmental factors, including nutrition and drugs, the possibility exists that one or more of the effects of a given 'hit' may be reversed by these means. Should this occur, accumulated effects will not reach clinical disease. LEARN: Latent early-life associated regulation; tLEARN: Transgenerational latent early-life associated regulation.

A model uniting environment, organism & generations: tLEARN

The original LEARN model explicitly unites 'environment', described in whole or part as 'envirome' [66,67], 'exposome' [67,68], and so on, and the organism 'as an information network' [63]. The envirome is essentially 'anything outside the organism that can influence its function through epigenetic changes'. It includes sociocultural effects (poverty, education and status) and historical events (warfare and economic cycles),

as well as nutrition and exposure to toxins, radiation or pathogens (sometimes called the exposome), and even elements of development and aging, which are not entirely governed by internal programs (Figure 2). From therapeutic and preventative standpoints, many aspects of the envirome are highly influential but of secondary usefulness, usually because of presumed difficulty inherent in controlling those particular elements. Two parts of the envirome that receive a great deal of medical attention are exposures to 'toxic' mate-

rials (including radiation) and nutrition. In particular, nutrition holds out potentially powerful ‘handles’ because it is one factor that could hypothetically be tailored and managed at the individual level.

An organism in interaction with the envirome (Figure 3) can be seen to exist on two levels. Physically, it is the information coding and transmitting molecules within an organism. These include nucleic acids (DNA and RNA) and the chemical modifications made to them (e.g., methylation and oxidation); the histones and their modifications (e.g., acetylation and phosphorylation); the proteome, which has its own primary amino acid sequence chemical modifications; and spatial (re)organization of any or all of these biochemical elements. An organism can also be modeled as an information network that ‘resides’ in the ‘media’ of the various molecules (and their 3D structures), and this changes over time, a highly dynamic system that includes both relatively stable (chromosomal DNA) and ephemeral (RNA, proteins and epigenetic markers) physical elements, together with factors that are not physical objects, but are how the objects are arranged at a given moment. It is constantly under modification in response to environmental influences. An organism can also be seen as the substrate of the envirome [66,67]. In addition, each individual organism (person) can exert influence to a greater or lesser degree upon its individual envirome and larger shared enviromes, thus influencing other organisms (people). Within LEARN, it is an environmental activity upon

the organism throughout the lifespan that gives rise to idiopathic disorders. Transgenerational (tLEARN) extends this concept across generations.

Nongenetic ‘intergenerational transmission’ of traits such as ‘harsh parenting’ [69] and domestic violence [70] is documented through purely behavioral explanations, as have behavioral prevention [71] or mitigation [72,73] of such intergenerational social etiology. Nevertheless, excesses of behaviorist presumptions and haste to draw simple environmental connections have, in the past, given rise to now thoroughly discredited claims, such as the ‘refrigerator mother’ etiology of ASD [74], the ‘aluminum hypothesis’ of AD [75] or the ‘schizophrenogenic mother’ of schizophrenia [76], among many other discarded overly enthusiastic environmental hypotheses. The failure of such discarded hypotheses has helped give rise to a greater caution with regard to any type of ‘intergenerational transmission’ of specific disorders (as opposed to specific behaviors) that does not rely entirely or primarily upon strict genetic inheritance.

The heritability of genetic conditions and risks is not currently questioned. It is not asked if it is possible for traits or conditions to be inherited, only to what degree a trait is heritable. Under the classic ‘central dogma’, that environmental influences could be inherited beyond those that manage to change germline DNA was unthinkable. Recent work has since introduced more flexibility in so-called purely biological traits. For example, glucose intolerance can be induced by neonatal overfeeding. This intolerance does not alter

Figure 2. The envirome. The envirome is essentially everything external to an organism, including, but not limited to nutritional factors (malnutrition vs nutrition); sociocultural and historical factors, such as economic and social status, famine and warfare; exposure to ‘chemicals’ and radiation; and the effects of existing through time (development and aging). Nutrition and exposure to ‘chemicals’ and radiation can be seen as a general ‘exposome’. Each of these can influence the others to some degree. Sociocultural factors exert very strong effects on the exposome, particularly in nutrition, but also in providing protection (or lack) against hazardous chemicals.

Figure 3. The organism as an information network. An organism includes multiple ‘information-containing and transmitting systems and molecules’, some of which may be inheritable. Broadly speaking, it can be seen as an interlocking network that includes the chromosomal and mitochondrial genomes (encoded by primary DNA sequences – mitochondrial not shown for reasons of space). The DNA of the genome plays host to some of the molecules that encode the epigenome. The epigenome consists of both modifications to individual DNA bases (usually cytosine or guanine) and modifications of the histone complex of the nucleosome (DNA omitted from histone complex diagram). Histone and DNA base modification can affect each other. Likewise DNA base modification can result in changes in the DNA primary sequence. The epigenome and genome together contribute to chromatin structure, and changes in chromatin structure can alter the likelihood of epigenomic modifications. Chromatin forms the basis for the RNAsome, which includes mRNA and regulatory molecules such as miRNA. The regulatory RNAs primary the processing of other RNAs, such as mRNA, and mRNA forms the template for translation to peptide primary sequences, which contribute heavily to the proteosome. The proteosome, itself, can modify any or all other levels, therein. The environment can, additionally, act upon any or all levels of the network, as well.

the DNA sequence, but it can be inherited [77]. Other specific instances of nongenetic inheritance of induced traits include paternal nutrition altering gene expression and health of offspring, particularly in adipose and pancreatic islet tissues and in expression of metabolic genes in general [78,79]. The CNS does not have a privileged immunity from nongenetic heritable alterations. Certain stress pathologies and responses have turned out to be epigenetic in nature and these changes are heritable [80,81]. Of particular interest, changes in brain function can be due to transgenerational epigenetic transmission [82], including neurobiological pathology [83–85] and

‘behavioral’ traits, such as aversion to acetophenone [86]. In ASD, for example, low paternal folate in diet [87], paternal age [42,88], grand paternal age [89] and paternal obesity but not maternal obesity [90] contribute to risk for ASD. While an argument may be made that effects such as grandpaternal age might be a genetic trait in that it might reflect a genetic propensity to reproduce later in life, linked to social traits common to ASD, factors such as paternal folate levels in diet would be more difficult to casually dismiss as ‘genetic’. More work needs to be done to determine specific genetic/epigenetic/combined mechanisms of these intergenerational observations.

The paternal connection is particularly interesting because maternal conditions could influence ASD risk through well-known pre/perinatal pathways and not nongenetic inheritance. The majority of work relating intergenerational nongenetic effects to a neuropsychiatric disorder has been in ASD. It has been the presumption that the important environmental effects in late-life disorders such as PD and AD occur within the patient's own lifetime. Likewise, little specifically intergenerational epigenomic work has been performed in earlier-manifesting conditions such as schizophrenia or major depressive disorder. Nonetheless, some potential connections have been drawn for schizophrenia [91], depression and glucocorticoid sensitivity [92], and PD [93].

In short, tLEARn treats each person as a 'unit' that can accumulate preclinical or subclinical 'hits' as described in the original LEARN model [63] and these changes can then be passed along to offspring along with purely genetic (DNA primary sequence) changes. It is the transgenerational accumulation of hits that ultimately determines a sporadic disease state. For many people, few, if any, significant transgenerational hits would accompany their conception or gestation. For conditions that develop late in life, transgenerational effects are likely to be swamped by lifetime effects. LEARN is not so much replaced by as continued by tLEARn. Specifically, two people may undergo a set of potentially transmissible hits in their lifetimes. Neither of them accumulates enough hits and/or their hits do not accumulate before a critical pathogenic developmental cutoff. However, they are passed along through epigenomic inheritance. The child of these two people may thus have 'preaccumulated' sufficient hits to be at significant risk for a disorder (Figure 4). It must be stressed that the hits need not produce symptoms or microphenotypes in either parent to have a transgenerational effect. For example, epigenomic alterations that could direct the processing of the Alzheimer's associated amyloid- β precursor protein (APP) away from the Alzheimer's related amyloidogenic path and toward the 'anabolic' or α -processing pathway could actually be neuroprotective in adulthood. However, this specific instance is even more complex. Evidence exists that the anabolic pathway, in excess, may contribute to autism in early life. Specifically, the sAPP α product of the nonamyloidogenic processing of APP is neuroprotective and neuroproliferative [94,95]. Significantly higher levels of sAPP α have been reported in samples from autistic children versus nonautistic children [96,97], and particularly high APP levels may particularly correlate with increased aggression in autistic subjects [98].

Evidence for intergenerational transmission of traits via epigenetics

Prenatal exposure to dangerous chemicals and toxins can produce disease or increase the chance of disease in the fetus. How these changes may persist over generations has yet to be fully elucidated. Prenatal exposure to vinclozolin, a pesticide, can lead to impaired fertility in the F3 generation of the patriline [99]. However, further work has proved less clear-cut, and the original result has been both confirmed and failed to confirm [100,101]. Nevertheless, similar generational effects have also been observed after exposure to jet fuels, plastics, and other environmental toxins during gestation. These exposures seem to induce permanent epigenetic changes in the germline, and these heritable changes lead to adult-onset disease in subsequent generations without any further exposure to the insult. What is particularly interesting is that this process can apply to behavioral disorders, such as addiction and resistance to addiction [102–104]. Paternal cocaine use in mice prior to having offspring alters expression of DNA methyltransferases (*DNMT*) 1 and 3a in the seminiferous tubules and may lead to alterations in development of female offspring [105]. Such alteration of DNMT levels may be a means of transgenerational epigenetics.

Generational transmission of traits leading to conditions related to obesity and metabolic disease has some evidentiary support but is not conclusively demonstrated [106]. Alternate scenarios include environmental exposure altering developing germ cells within the developing fetus that are not manifest as epiphenotypes in F1 generation and effects induced into the fetus itself, which would be transmitted to the germline, affecting the next generation [107]. Recent evidence from mouse studies has shown that exposure of parents to environmental factors can have a profound effect on the changes in insulin sensitivity seen in Type 2 diabetes in offspring [108]. Generational effects have been seen in animal studies during which mice are reexposed to environmental stressors throughout several generations. Epigenetic models of obesity and the metabolic syndrome propose a programmed dysregulation of body weight in mice [109].

The experiences of one generation can seemingly alter the behavior of the subsequent offspring. The experience of maternal separation during early development altered DNA methylation levels in the brains and sperm of offspring and grandoffspring [110]. It has also been shown that there is a period of time during early gestation when stressors upon the dam, such as 36 h constant light, 15 min of predator odor, novel objects in the cage, overnight 5 min restraint in a 50-ml conical tube, novel white noise overnight, multiple cage

Figure 4. Intergenerational effects of enviromes on organisms: transgenerational latent early-life associated regulation. A simple one-generation pedigree is presented, in which the maternal envirome (Envirome_M) acts upon the initial maternal parent (Organism_{M0}) any number of times until time of reproduction/parturition (Organism_{Mn}), at which it makes its genetic and epigenetic contributions to the offspring (Organism_{F0}). The paternal counterparts (Envirome_P, Organism_{P0} and Organism_{Pn}) likewise contribute in a similar fashion. Although not shown, the maternal contribution includes additional material, such as mitochondria, which would also be subject to environmental influence. The offspring, from conception, is then affected by the filial envirome (Envirome_F), which would include prenatal and perinatal conditions. Ultimately, this would give rise to the offspring at time 'n' (Organism_{Fn}), which could suffer a neuropathological condition. Although presented as distinct, the various enviromes can easily overlap to greater or lesser degree, and envirome–organism interactions are a multiple events.

changes and water-saturated bedding overnight can lead to dysmasculinization in the male offspring that recurs for several generations, and this is transmitted from the prenatally stressed males through their male offspring [111]. In adult male rats, increased anxiety, abnormal behavior and elevated corticosteroid levels were observed in both male and female offsprings of male mice after these mice underwent chronic social defeat paradigm. All offsprings were sired by *in vitro* fertilization. This permitted sperm to be collected from the defeat-exposed sires both before and after their exposure. Offspring from postdefeat-exposed sires had higher levels of anxiety/abnormal behavior and corticosteroids both in comparison to the same sires (predefeat) and no-exposed sires [112].

The role of these epigenetic changes on social behavior is critical for the understanding of these traits across generations, especially as related to disorders such as ASD and schizophrenia. These changes may also highly influence parenting style choices of mothers for future generations. Germline effects have been observed in fathers, hinting to their role in transmitting influence epigenetically to their offspring perhaps making up for a lack of parental care and influence often provided by the mother [113]. Transmission of gestational programming effects occurs in subsequent generations in the absence of continued adverse environmental exposures [114]. For example, the 'Dutch Famine' (1944–1945) cohort showed that prenatal exposure to famine resulted in hypomethylation of *IGF2* gene in whole blood, and

hypermethylation of two obesity-related nonimprinted genes (tumor necrosis factor, leptin) compared with same-sex siblings who had not been exposed to the famine [115,116]. In addition, increased adiposity was observed in the offspring of prenatally undernourished

fathers from that cohort [117]. Understanding the mechanism of how traits are maintained and transmitted in the germline is the key to developing strategies to prevent environmental exposures from producing a disease phenotype across generations.

Executive summary

Genomic variations

- Small variants, copy number variants and rare variants do not add up to explain the cases of sporadic diseases.
- Most SNPs have little clinical value.
- Copy number variants and other structural variants contribute to genetic variation, and they are frequently found in both healthy people and those with 'sporadic' diseases.
- Rare genetic variants do not provide an explanation of over 90% cases of bipolar disorder, schizophrenia, or autism spectrum disorder.
- Genetic models can be greatly supported and expanded by explicit mechanistic explanations of gene–environment interaction.

Genetic variation on its own does not explain all pathogenesis of 'sporadic' disorders

- In many disorders with known genetic components, such as Alzheimer's disease, cases that can be explained solely by genetic mutation are a minor fraction.
- There is an important role played by epigenomic factors in conditions as diverse as Alzheimer's disease, Parkinson's disease, schizophrenia and even suicide.
- Epigenomic markers include modifications of DNA and of chromatin histones.
- Epigenomic contributions to neurobiological disorders may actually reach across generations.

Latent early-life associated regulation unites environment & gene expression

- For many sporadic disorders, the body accumulates 'hits' (that may include some genetic predispositions).
- No single hit is sufficient to cause disease.
- Each is individually latent.
- Many hits are of environmental origin and are 'recorded' in the organism through epigenomic markers.
- If a sufficient number of 'correct' hits occur before a critical developmental/aging threshold, the organism will develop the corresponding disorder

A model uniting environment, organism & generations: transgenerational latent early-life associated regulation

- In interaction with the environment, an organism exists on two levels.
- These are the information-coding and transmitting molecules and an information network that 'resides' in the 'media' of the molecules.
- An organism can also be seen as the substrate of the envirome.
- extends this across generations.
- Changes in brain function, such as aversion to acetophenone, can be due to transgenerational epigenetic transmission. In autism, for example, low paternal folate in diet, paternal age, grand paternal age and paternal obesity but not maternal obesity contribute to risk.

Evidence for intergenerational transmission of traits via epigenetics

- Exposures induce permanent epigenetic changes in the germline.
- Heritable changes lead to adult-onset disease in subsequent generations without further exposure to insult.
- This can apply to behavioral disorders, such as addiction and resistance to addiction.
- Mouse studies show that exposure of parents to environmental factors can have a profound effect on the changes in insulin sensitivity seen in Type 2 diabetes in offspring.
- The experiences of one generation can alter the behavior of subsequent offspring.
- Experience of maternal separation during early development altered DNA methylation levels in the brains and sperm of offspring and grandoffspring.

Conclusion & future perspective: research & treatment with transgenerational latent early-life associated regulation

- A longitudinal epigenome/envirome-wide association study would unite genetic sequence, epigenomic markers, environmental exposures, patient personal history at multiple time points and family history.
- Cohorts would include representatives of at least two generations, preferably more.
- The possibility exists that, should environmentally induced, inherited epigenomic 'mis-markings' increase risk for disorders in offspring, supplementing diet while avoiding repeating parental exposures may permanently reduce inherited risk.

Conclusion & future perspective: research & treatment based on the tLEARn concept

Testing tLEARn will require longitudinal, synthetic approaches. GWAS are end point assays that compare genetic sequences between diseased and nondiseased individuals. Several assays currently exist, which address parts of the question, but none consider the whole picture, particularly not in relation to changes over time. A longitudinal epigenome/envirome-wide association study (LEWAS) would unite genetic sequence, epigenomic markers, environmental exposures, patient personal history taken at multiple time points and family history. Ideally, cohorts would include representatives of at least two generations, preferably more. Several studies have partially implemented the LEWAS concept. Mouse studies have measured changes in specific gene-associated epigenetic changes to environment and concomitant changes in expression for those specific genes [118–120]. Targeted epigenomic surveys have been performed on human populations, comparing ‘end point’ differences in epigenomic markers and disease [63,121–123]. However, no study method currently unites multiple measurements over time with human populations and an envirome/organism-wide approach. Large-scale epigenomic surveys are currently underway, but these are end point, focused, without tracking changes in the epigenome [124,125]. The novel element of LEWAS is its longitudinal approach, attempting to measure, rather than infer *post hoc*, disease-critical organismal changes and relates those to previous environmental influences. LEWAS poses challenges in terms of scope and cost, particularly the need for large sample sizes in the face of unknown later development of disease and multiple sampling times. Multiple longitudinal studies have been done or are ongoing for conditions such as AD [126–129], PD [130,131], ASD [132–134], bipolar disorder [135,136], psychotic disorders [10,137] and other conditions [137], although not all are biochemical or epigenetic studies. Modern communication (especially the advent of social media and internet-based rating systems) has made these studies far more feasible than in the past. Methods exist to begin studies with very large sample sizes and reduce them to a ‘hypothesis relevant’ sub-sample [138]. For disorders of the CNS or other vital organs, direct sampling will not be possible. However, proxy tissues may be developed. For example, olfactory neuroepithelial cells are easily accessed and

can be repeatedly sampled with safety. These have been adequate proxies for other neurological studies [139]. Also stem cell technology has advanced such that neuronal cells may be developed that reflect the genetic (and possibly epigenetic) characteristics of the donor. These can be modified with CRISPR-Cas 9-based methods.

Of course, one exciting possibility of tracing epigenomic antecedents to disorders is new treatment avenues, including the possibility of treatments that may not need to be lifelong. In particular, aberrant hypomethylation of DNA could potentially be reversed and this reversal could be maintained by means of avoiding high level or chronic exposures to materials such as pesticides and by eating a diet rich in materials like S-adenosyl-methionine (SAM). Dietary SAM supplementation via apple juice concentrate has been shown to reverse aberrant hypomethylation in AD-model mice [140]. Likewise, such supplementation also reversed cognitive deficits and reoriented DNA methylation in dietary-induced DNA hypomethylated mice [141]. It is worthwhile to explore the possibility that if specific environmentally-induced, inherited, epigenomic ‘mis-markings’ increase risk for sporadic disorders in offspring, perhaps supplementing diet while avoiding repeating parental exposures could permanently reduce inherited risk. Other ongoing studies exploring the epigenetics of dementias would provide potential preventative and therapeutic strategies [141]. Finally, as manifestations of the gut influence on behavior and diseases are beginning to be understood, future directions would further investigate the influence of gut microbiota [142] on tLEARn.

Financial & competing interests disclosure

The work was supported by grants from the National Institute on Aging, NIH R01-AG051086 and R21-AG042804; and Indiana Clinical & Translational Sciences Institute (ICTSI) and Indiana Spinal Cord and Brain Injury Research Fund (ISCBIRF); P30 AG010133 Indiana Alzheimer Disease Research Center, and R21 sub-award from Purdue University to DK Lahiri. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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