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Neurobiology of Aging 32 (2011) 1188–1191

NEUROBIOLOGY OF AGING

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Open peer commentary

Toward an integrated genetic and epigenetic approach to Alzheimer's disease

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Received 22 October 2010; accepted 23 October 2010

Abstract

Epigenetics is the study of mitotically heritable, but reversible, changes in gene expression brought about principally through alterations in DNA methylation and chromatin structure. The comprehensive review by Mastroeni et al. (Mastroeni, D., Grover, A., Delvaux, E., Whiteside, C., Coleman, P., Rogers, J., 2010. Epigenetic mechanisms in Alzheimer's disease. Neurobiol. Aging, doi:10.1016/j.neurobiolaging.2010.08.017) in this issue describes mounting evidence for an involvement of epigenetic alterations in the etiology of Alzheimer's disease (AD), highlighting the potential of epigenomic approaches for uncovering novel molecular pathways involved in pathology. Here, we briefly describe some methodological issues related to epigenomic studies using postmortem brain tissue in Alzheimer's disease, and argue for an integrated genetic-epigenetic approach to disease etiology.

Keywords: Epigenetics; Alzheimer's disease; Genetics; Allele-specific DNA methylation; Postmortem brain samples

1. Looking "above" the DNA sequence: epigenetics in Alzheimer's disease

Although the neuropathological changes associated with Alzheimer's disease (AD) have been well characterized, there is still considerable debate regarding the underlying etiology of the disorder and the precise mechanism(s) behind disease progression. Given the high heritability estimates for AD (Gatz et al., 2006), much research has focused on uncovering a genetic contribution to the disorder. While autosomal dominant mutations in 3 genes (*APP*, *PSEN1*, and *PEN2*) can explain early onset familial AD, these account for only 5%–10% of the total disease burden. Most cases of AD are late onset (>65 years), non-Mendelian, and highly sporadic, with susceptibility attributed to the action of highly prevalent genetic variants of low penetrance. To date, the only widely replicated genetic risk for late onset AD remains the ε4 allele of the apolipoprotein E gene (*APOE*), accounting for about a third of the population-attributable risk for the disorder (Hardy, 2006). Although there have been numerous studies attempting to elucidate the underlying mechanism for this association, precisely how apolipoprotein E4 (apoE4) influences AD onset and progression has yet to be shown. Recent advances in genomic technology in conjunction with the collection of large sample cohorts have heralded the advent of genome-wide association studies (GWAS) aimed at identifying novel genetic risk factors (Gandhi and Wood, 2010). While a number of susceptibility loci (e.g., *CLU, CR1*, and *PICALM*) have been uncovered via genome-wide association studies (GWAS) (Harold et al., 2009; Sleegers et al., 2010), these account for only a small proportion of attributable risk and the mechanism behind their action remains unknown. Despite considerable research effort, therefore, we are still a long way from realizing the postgenomic promises of novel diagnostic and therapeutic strategies for AD; currently there is no cure for AD and the available treatments (pharmaceutical, psychosocial, and care-giving) provide only small symptomatic benefits.

As highlighted in the comprehensive review by Mastroeni and colleagues (Mastroeni et al., 2010), there is now growing recognition that epigenetic mechanisms are likely to be important in the etiology AD. Epigenetics refers to the reversible regulation of various genomic functions, occur-

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doi:10.1016/j.neurobiolaging.2010.10.021

ring independently of DNA sequence, mediated principally through changes in DNA methylation and chromatin structure (Jaenisch and Bird, 2003). Epigenetic processes are essential for normal cellular development and differentiation, and allow the long term regulation of gene function through nonmutagenic mechanisms (Henikoff and Matzke, 1997). Because epigenetic processes play a critical role in cellular development and function, aberrant DNA methylation and/or histone modifications are hypothesized to be involved in a diverse spectrum of human pathologies including cancer (Jones and Laird, 1999), congenital imprinting disorders (Feinberg, 2007), and a number of complex chronic disease phenotypes including AD (Chouliaras et al., 2010; Mastroeni et al., 2010). Elucidating genomic patterns of epigenetic modifications and the factors influencing them will have important implications for understanding the causes of human health and disease. Furthermore, the discovery of epigenomic dysfunction associated with AD would be particularly exciting given the dynamic regulation of epigenetic phenomena; unlike pathogenic DNA sequence mutations, epigenetic disruption is potentially reversible, and thus a realistic target for pharmacological intervention. It is plausible that the discovery of specific epigenetic changes associated with AD will expediate the development of novel therapies for AD. This notion is supported by experiments on a mouse model of neurodegeneration that demonstrates how histone deacetylase (HDAC) inhibitors can stimulate the recovery of learning and memory via chromatin remodeling (Fischer et al., 2007).

2. Considerations for epigenetic studies in AD

The rapid growth in epigenetic research, combined with new high-throughput technologies for epigenomic profiling (Laird, 2010), promises to revolutionize our understanding about the molecular changes associated with complex disease. While it is now possible to systematically profile DNA and histone modifications across the genome, little empirical research has directly studied the role of epigenetic factors in AD, especially taking an unbiased, genome-wide approach across multiple brain regions. Given the tissue-specificity of epigenetic marks, and since it is not yet possible to perform in vivo epigenetic studies, research using postmortem tissue from the brain is particularly important for directly interrogating the site of AD manifestation, where molecular changes are most likely to be functionally relevant. While numerous studies have examined AD-associated gene expression changes in postmortem brain samples, epigenetic studies are in their infancy. In taking this research forward, it is important to recognize the numerous methodological and logistical issues related to epigenomic studies using brain tissues (Pidsley and Mill, 2011). For example, there have been very few studies specifically investigating the effects of pre-, peri-, and postmortem conditions on epigenetic status in postmortem brain tissue. Additionally, little is known about how factors such as age, sex, medication, and other environmental exposures influence epigenetic patterns, so clinical tissue samples should be matched as carefully as possible for all potential confounding variables.

The development of methods to assess epigenetic marks in specific cell populations within the brain is likely to be critically important in AD. Tissues like the cortex, for example, consist of numerous different types of neuronal and glial cells, and many brain-expressed genes are only transcribed in a subpopulation of neurons (Nelson et al., 2006). Therefore, the detection of cell type-specific epimutations is likely to be difficult unless the epigenetic profile of specific cells can be investigated. By using techniques such as laser capture microdissection (LCM), we may be able to specifically isolate glial and neuronal cell populations to also examine whether observed epigenetic changes are a consequence of the neurodegenerative processes associated with AD (i.e., differential cell death and gliosis) or the result of epigenomic changes in the remaining neurones.

High-throughput methods for profiling epigenetic marks are still in development, and no consensus has been reached about the optimal research strategy. Even the most sophisticated method has its own limitations, meaning that it is vitally important to use standard quality control procedures, to control for multiple testing and carefully verify any disease-associated changes using an alternative approach where necessary. Epigenetic studies of the brain are also limited by insufficient knowledge about the "normal" epigenetic patterns that characterize different brain regions and cell types, although it is hoped that current mapping initiatives such as those supported by the National Institutes of Health (NIH) Epigenomic Roadmap (Bernstein et al., 2010), that aim to catalogue patterns of epigenetic variation across different cell and tissue types, will be useful in this regard. Furthermore, despite the tissue-specific nature of the epigenome, there is increasing evidence from other disorders that many epimutations are not limited to the affected tissue or cell type, but can also be detected in other tissues. To date, however, no study has attempted to systematically map epigenetic variation between specific brain regions and correlate these with DNA methylation in peripheral tissues from the same individual. Understanding the relationship between brain and peripheral epigenetic signatures could pave the way for future large scale epigenomic studies of AD and other neuropsychiatric conditions, given the paucity of high quality postmortem brain tissue. Identifying peripheral epigenetic biomarkers correlated with disease-associated changes occurring in the brain could impact greatly upon the clinical diagnosis, prognosis, and treatment of AD; the current histopathological markers used to definitively identify AD (i.e., plaques, tangles, and brain atrophy) can only be evaluated definitively in postmortem brain tissue.

3. Allelic effects on DNA methylation: implications for an integrated genetic-epigenetic approach to AD

We have recently argued for the adoption of an integrative etiological research paradigm based on the combination of genetic and epigenetic data (Meaburn et al., 2010). Of particular relevance to the etiology of complex disease phenotypes such as AD is increasing evidence for the widespread occurrence of allele-specific epigenetic marks. Outside of classically imprinted autosomal regions, and regions subject to X-chromosome inactivation in females, DNA methylation is generally assumed to be complementary on both alleles. Recent research, however, has shown that this is not necessarily the case with the discovery that allele-specific methylation (ASM) is a common feature across the genome (Hellman and Chess, 2010; Kerkel et al., 2008; Schalkwyk et al., 2010; Shoemaker et al., 2010). A key observation is that the majority of ASM is associated with genetic variation in *cis*, although a noticeable proportion is also non-*cis* in nature and mediated by parental origin at regions not previously believed to be imprinted. It is plausible that non-*cis* ASM could contribute toward the "missing heritability" of complex diseases, rendering certain loci hemizygous and masking the direct association between genotype and phenotype. ASM appears to be both quantitative, characterized by subtle skewing of DNA methylation between alleles, and heterogeneous, varying across tissues and between individuals. These observations have important implications for genetic studies of apparently heritable disorders such as AD; while *cis*-mediated ASM provides a functional consequence for noncoding genetic variation, heterogeneous and quantitative ASM complicates the identification of disease-associated loci (Meaburn et al., 2010). Because epigenetic processes may be influenced by a range of external environmental factors including diet, toxins, drugs, and stress (Dolinoy and Jirtle, 2008), the observation that polymorphisms can exert an effect on gene function via epigenetic processes occurring in *cis* suggests a common pathway behind both genetic and environmental effects and a potential mechanism for gene-environment interaction.

4. Future research directions in AD epigenetics

Technological advances in epigenomic profiling methodologies mean that it may soon be feasible to economically map the epigenome at single base pair resolution in large cohorts of samples; the first reference single base resolution map of the methylome was recently published for 2 human cell lines, providing detailed information about the extent and location of methylated loci (Lister et al., 2009). Moving forward, it will be important to establish cause and effect in epigenetic studies of AD; disease-associated epimutations may arise prior to the illness and contribute to the disease phenotype or could be a secondary effect of neurodegeneration, or even the medications used to treat AD. Furthermore, maximum information will be obtained from studies integrating epigenomic information with genomic, transcriptomic, and proteomic data obtained from the same samples. Ultimately, epigenetic research holds huge promise for the development of novel diagnostic and therapeutic measures for AD and other neuropsychiatric conditions.

Disclosure statement

No conflicts of interest exist.

Acknowledgements

This work was supported by NIH grant AG036039.

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